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THE EXCRETION RATE OF UREA IN THE TOXEMIAS OF PREGNANCY

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Following the appearance, in 1909, of the work of Ambard and his collaborators on the rate of urea excretion, a considerable literature has accumulated upon the subject, most of the investigations having been undertaken in the hope of establishing an index of urea excretion which might serve as a criterion of the functional capacity of the kidney.

Ambard maintained that the urea excretion rate is proportional to the square of the concentration of urea in the blood, other factors remaining constant. Thus, according to his first law, with a constant concentration of urea in the urine, $\frac{D}{B^2} = K$, D being the amount of urea excreted in 24 hours and B the urea concentration in the blood.

According to his second law, when the urea concentration in the blood is constant, the rate of urea excretion is inversely proportional to the square root of the urea concentration in the urine, viz., $K = D\sqrt{C}$, C being the urea concentration in the urine.

In 1914, Marshall and Davis stated that in normal animals, drinking freely of water, the urea excretion rate is directly proportional to the concentration of urea in the blood. In 1915, McLean devised the formula

$$I = \frac{\text{Gm. urea per 24 hrs.} \sqrt{\text{gms. urea per liter of urine}} \times 8.96}{\text{Wt. in kilos} \times (\text{gms. urea per liter of blood})^2}$$

and stated that when $K = 0.080$, the standard normal calculated according to Ambard, I (the index of urea excretion) $= 100$. In 1916, Addis and Watanabe concluded from experiments on young healthy adults that "the normal kidney under constant conditions is characterized by the possession of a high degree of constancy of function." In their experiments urea in large doses was administered in an attempt to produce "kidney fatigue."

but they were unable to bring about such a condition. In general it may be said that the work of Addis and his co-workers indicates that the rate of urea excretion is fairly dependent on the urea concentration in the blood.

In 1921 Austin, Stillman and Van Slyke published their formula $K = \frac{D}{B\sqrt{V}\,W}$, where D is the urea output expressed in grams per 24 hours, B the blood urea, in grams per liter, V the volume of urine in liters per 24 hours, W the body weight in kilograms, and K the excretory constant. They found that K had a value of 7.5 ± 3 for normal man. Moreover, they demonstrated that there exists an "augmentation limit" in the volume output of urine. This limit lies between an output of 2.5 and 6 liters per 24 hours, and is in excess of the rate usually encountered except where large amounts of fluid had been ingested. Their formula, therefore, holds true only where the volume of urine output lies below this "augmentation limit," which varies for different individuals; whereas, should the amount of urine excreted exceed this limit, it will become necessary to modify the formula. They further believe that there is a similar augmentation limit in the blood urea concentration, this belief being based in great part upon the work of Addis, already referred to.

Addis and Drury later developed the index $\frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 c.c. of blood}}$, and stated that under certain special conditions it remains fairly constant, over wide ranges of blood-urea concentration. They emphasize the fact, however, that there are many factors other than blood-urea concentration which may influence the rate of urea excretion.

From autopsy study and clinical observation it is evident that the kidneys play an important rôle in certain of the toxemias of pregnancy, although we still lack a satisfactory method of differentiating between the several varieties. It consequently occurred to us that it might be of interest to study the rate of urea excretion in the different types, in the hope of ascertaining whether characteristic variations in the functional capacity of the kidney could be demonstrated in these conditions, and possibly be utilized in clinical work.

The accompanying tables give our results. We have studied the conditions obtaining in the normal non-pregnant and in the gravid woman, as well as those occurring in neurotic vomiting of pregnancy, nephritic toxemia, pre-eclamptic toxemia and in actual eclampsia. We have tried to control all variable factors as far as feasible. All determinations, with the exception of those given in Table I, were carried out with the patients in bed and on strictly regulated diet, as noted in the tables. Specimens of blood were taken in the morning before breakfast, samples of one hour's urine were obtained from 10 A. M. to 11 A. M., the 24-hour urine specimens collected with the utmost care from 6 A. M. of one day to 6 A. M. of the following day, and kept in the ice-box.

The urea nitrogen in the urine was determined by the Van Slyke-Cullen method and that in the blood by the Van Slyke-Cullen modification of the Marshall method; while the non-protein nitrogen was determined according to the directions of Folin and Wu.

It seemed advisable to study the Index of Addis and Drury, as well as the formula of Austin, Stillman and Van Slyke. In our tables the former is referred to as A. D. I. (representing the ratio $\frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 c.c. of blood}}$ and the latter as K, where $K = \frac{D}{B\sqrt{V}\,W}$, D, B, V and W being expressed in the units explained above.

We have worked out a third ratio which we call X. This ratio expresses the relation between the blood urea nitrogen (in mgms. per 100 c.c. of blood) and the urine urea nitrogen (expressed as a percentage of the total nitrogen in the urine). In order to deal with whole numbers, we have multiplied this ratio by 100. Our data seem to indicate that the urea nitrogen in the urine, when expressed as a percentage of the total nitrogen in the urine, could be associated with clinical variations.

It is for this reason that we have tried to use this percentage figure in our ratio. Designating the blood urea nitrogen in mgms. per 100 c.c. of blood as B, the urea nitrogen in gms. per 24 hours' urine as D (as in the formula of Austin, Stillman and Van Slyke), and the total nitrogen in gms. per 24 hours' urine as T. N., one may calculate our index as follows:—

$$X = \frac{B}{\frac{D}{T.N.} \times 100} \times 100$$

$$= \frac{B \times T.N.}{D}$$

In all the indices urea is expressed as urea nitrogen, because, in the index X, we employ the figure representing the urea nitrogen as a percentage of the total nitrogen in the urine.

Discussion

In all our tables the index of Addis and Drury (A. D. I.) varies between wide limits. We ascribe this to the fact that the amount of urine excreted by any individual in one hour varies greatly. The 24-hour urine output, on the other hand, is a more constant quantity. The nurses on our wards have been specially instructed regarding the collecting of 1-hour and 24-hour urine specimens, yet we find that the volume excreted in 1 hour is rarely constant for the same individual.

Because of the lack of uniformity in the values we have obtained for the A. D. I., we shall not further discuss this index.

Normal Women.—As will be seen from Tables I and II, the index of Stillman, Austin and Van Slyke, i.e. K, has fairly constant values, with an average of 4.1 for the

TABLE I.

NORMAL NON-PREGNANT WOMEN.

Number	Date	Age	Color	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine Urea N. in gms.	24-hour Urine Urea N. in gms.	A. D. I.	K	X
I	1/25/24	26	W	116/70	54.5	35.3	20.08	31	811	0.283	7.23	14.1	5.4	25.0
II	1/25/24	26	W	110/68	65.4	33.0	19.15	27	1207	0.194	6.94	10.1	4.1	24.9
III	1/25/24	26	W	110/70	56.8	33.3	21.95	35	965	0.258	7.19	11.75	4.4	27.9
IV	1/25/24	24	W	118/60	52.7	39.5	28.02	360	2020	0.709	7.61	25.2	2.6	29.2
												Average—	4.1	26.5

TABLE II.

NORMAL PREGNANCY.

Number	Date	Age	Color	Duration of Pregnancy	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine Urea N. in gms.	24-hour Urine Urea N. in gms.	A. D. I.	K	X
A	1/16/24	19	B	Term	108/90	59.0	23.4	10.74	70	1030	0.23	5.47	21.4	6.5	14.6
B	1/16/24	23	W	Puerp.	90/65	51.1	29.1	14.01	50	1310	0.29	9.37	20.69	8.2	18.4
C	1/16/24	19	W	"	112/80	67.2	28.2	11.68	55	550	0.21	3.67	17.97	5.2	15.9
D	1/16/24	19	W	"	108/70	53.5	31.6	13.54	160	1660	0.497	7.747	36.7	6.1	17.4
E	1/16/24	24	B	Term	120/75	57.0	27.3	12.61	22	682	0.16	4.85	12.68	6.2	15.9
													Average—	6.4	16.4

TABLE III.

NEUROTIC VOMITING OF PREGNANCY.

Number	Date	Age	Color	Duration of Pregnancy	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine Urea N. in gms.	24-hour Urine Urea N. in gms.	A. D. I.	K	X	Remarks
19	9/18/23	20	B	3½ mos.	125/88	76	37.5	14.48	55	1095	0.33	4.11	22.79	3.1	28.8	
32	10/12/23	26	W	3 mos.	112/65	48	37.5	14.01		600		3.67		4.9	29.0	
	10/15/23					48.2	27.3	9.34		360		1.12		2.9	32.7	
	10/19/23					49	30.6	7.47		3200		5.05		5.4	13.4	Completely cured
													Average—	4.1	26.0	

TABLE IV.

NEPHRITIC TOXEMIA.

Number	Date	Age	Color	Duration of Pregnancy	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine Urea N. in gms.	24-hour Urine Urea N. in gms.	A. D. I.	K	X	Remarks
3	5/ 3/23	20	W	7½ mos.	180/100 110/70	64.7	35.3	17.28	130	1200	0.70	5.20	40.5	3.4	24.9	With convulsions. Milk Diet
21	9/22/23	41	W	Term	200/130 120/75	81.5	32.6	9.81	200	1400	0.57	4.02	58.1	3.9	17.0	With Convulsions. Milk Diet
	9/25/23					79.1	37.5	25.22		1210		4.49		1.8	34.2	With Convulsions. Milk Diet
	10/ 1/23					75.0	43 6	18.21		2910		8.81		3.3	23.1	With Convulsions. Milk Diet
	10/10/23					68.0	40.0	20.55		1250		2 55		1.3	25.4	With Convulsions. Milk Diet
6	5/ 7/23	39	B	Term	140/90 118/78	53 1	32.8	10.27		1460		2 51		2.8	18.7	Without Convulsions. Milk Diet
7	5/ 8/23	39	W	Puerp.	155/85 110/65	72.2	28.2	11.8	370	805	1.98	4.45	167.7	4.9	19.3	Without Convulsions. Milk Diet
9	5/ 9/23	28	W	7 mos.	98/50	57.2	20 0	11.68	105	2470	0.26	3.12	22.3	2.2		Without Convulsions. Milk Diet
	6/ 8/23			8 mos.	115/60	59.0	18.7	11.68	70	310	0.19	1.13	16.2	2.3		Without Convulsions. Milk Diet
16	8/18/23	30	W	Term	190/120 160/96	65.5	35.7	17.28		2620		4.02		1.8	21.8	Without Convulsions. Milk Diet
	8/19/23					65.5	31.2	14.94		600		2.02		2.2	19.1	Without Convulsions. Milk Diet
40	11/28/23	17	W	7 mos.	176/115 140/108	53 0	31.6	20.78	220	1920	0.179	2.53	8.61	1.2	26.9	Without Convulsions. Milk Diet
42	12/12/23	35	B	9 mos.	250/165 140/100	100.1	40.0	29.42	200	4200	0.261	6.30	8.87	1.0		Without Convulsions. Milk Diet
	12/29/23			Puerp.		78.8	33.9	16.81	160	1160	0.423	4.85	25.1	3.0	20.2	Without Convulsions. Milk Diet
													Average—	2.4	22.8	

non-pregnant and 6.4 for the pregnant woman. It is, however, quite clear from these tables that our index, X, is a more constant figure. The average for X is 26.5 in the non-gravid and 16.4 in the gravid female. It is mainly the decrease in the blood urea concentration in pregnancy that accounts for the marked decrease in X, from 26.5 to 16.4.

Neurotic Vomiting of Pregnancy.—For both K and X the values are approximately the same as in the non-pregnant woman. As the patient improves, these figures approach those encountered in normal pregnancy. Our series of cases in this group is too small to permit us to draw definite conclusions.

Nephritic Toxemia.—In this complication of pregnancy there is a marked disturbance in both indices, K decreasing to an average of 2.4, and X increasing to an average of 22.8.

Pre-eclamptic Toxemia.—In this condition K has an average value of 3.3, although it fluctuates between wide

TABLE V.

PRE-ECLAMPTIC TOXEMIA.

Number	Date	Age	Color	Duration of Pregnancy	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine Urea N. in gms.	24-hour Urine Urea N. in gms.	A. D. I.	K	X	Remarks
5	5/ 3/23	20	B	Term	175 115 / 105 73	75.9	19.6	6.54	260	1600	0.24	3.04	36.7	4.2		Milk Diet
	5/ 8/23					61.5	24.0	7.94		2300		4.63		4.9		Milk Diet
12	6/ 7/23	40	B	7½ mos.	180 120 / 100 80	67.8	18.6	10.70	370	1600	0.83	3.14	77.5	2.8		Milk Diet
13	6/ 7/23	21	B	Term	160 125 / 110 95	71.1	21.6	12.61	140	1250	0.70	4.64	32.3	3.9		Milk Diet
17a	8/30/23	18	W	8½ mos.	160 120 / 105 78	57.0	35.7	33.16	230	1520	0.82	6.07	24.4	2.0		Milk Diet
b	9/14/23					56.0	35.7	25.22		1010		1.98		1.0	36.3	Milk Diet
c	9/12/23					52.0	38.5	20.55	200	3710	0.54	7.53	26.2	2.6	25.0	Milk Diet
22	9/22/23	18	W	Term	178 110 / 100 70	59.0	53.6	13.54	550	1550	1.48	4.50	109.3	3.5	25.7	Milk Diet
	10/ 5/23		W	Term	178 110 / 100 70	53.0	34.5	16.35		1150		5.02		4.0	24.6	Milk Diet
25	9/27/23	35	W	Term	170 120 / 91 70	85.5	38.9	18.60		2450		13.30		4.9	24.2	Milk Diet
35	10/16/23	23	W	Term	160 118 / 95 80	58.0	35 3	14 01		900		2.30		2.3	23.1	Milk Diet
	10/19/23	23	W	Term	160 118 / 95 80	57.0	39.5	17.75		2000		13.32		7.0	24.9	Milk Diet
													Average—	3.6	26.2	

limits, 1.0 to 7.1. The index X, on the other hand, is more constant. Its average value is 26.2.

Eclampsia.—In true eclampsia K is again low, while X is greatly increased, the average results being 2.2 and 28.7 respectively. Here the two indices vary to approximately the same extent.

From a comparison of the values for the two indices, K and X, in the different tables, it is evident that K varies over wider ranges than does X, although both show marked changes in the toxemias of pregnancy.

In an attempt to differentiate between the different varieties of toxemias, we may say that in the nephritic type X has a slightly higher value (22.8) than in normal pregnancy (16.4). In pre-eclamptic toxemia X is further increased (26.2), while in eclampsia it has its highest value (28.7).

CONCLUSIONS

1. We have been unable to obtain uniform results for the urea excretion rate by means of the index of Addis and Drury, which is $\frac{\text{Urea in 1 hour's urine}}{\text{Urea in 100 c.c. of blood}}$. This we ascribe to the fact that the volume of urine excreted in one hour may be a very variable quantity.

2. The index of Stillman, Austin and Van Slyke, $K = \frac{D}{B\sqrt{V}\sqrt{W}}$, gives fairly constant results. It manifests marked changes in the toxemias of pregnancy.

3. The index we have proposed, viz. $X = \frac{B \times T.N.}{D}$, affords in general more constant values than the index of Stillman, Austin and Van Slyke.

4. In the toxemias of pregnancy there is a marked disturbance in the value of X. The average value of X in our tables, is as follows:—

TABLE VI.

ECLAMPSIA.

Number	Date	Age	Color	Duration of Pregnancy	Blood pressure range	Weight in kilos	N. P. N. in mgms. per 100 cc. of blood	Blood urea N. in mgms. per 100 cc. of blood.	60-min. Urine Volume in cc.	24-hour Urine Volume in cc.	60-min. Urine N. in gms.	24 hour Urine N. in gms.	A. D. I.	K	X	Remarks.
14	6/13/23	16	B	Term	170/110 100/95	70.0	25.0	14.94	120	550	0.78	2.07	52.2	2.2	21.1	Milk Diet
15	8/24/23	20	W	Term	160/110 110/70	72.7	34.9	17.75		940		2.81		1.9	31.5	Milk Diet
29	10/ 7/23	29	W	Term	182/102 116/74	67.0	30.7	14.01		950		2.81		2.5	20.5	Milk Diet
	10/11/23	29	W	Term	182/102 116/74	65.0	40.0	20.08		5000		6.70		1.9	32.8	Milk Diet
38	12/ 3/23	27	B	8½ mos.	190/120 105/70	63.0	40.6	28.49	220	2100	0.308	7.36	10.81	2.3	36.1	Milk Diet
	12/ 8/23	27	B	8½ mos.	190/120 105/70	61.5	35.9	24.75	175	4475	0.476	9.85	15.1	2.4	34.3	Milk Diet
44	1/24/24	19	W	Puerp.	200/110 125/70	52.0	45.0	16.81	240	2540	0.365	5.41	21.71	2.8	24.5	Milk Diet
45	1/22/24	16	B	Term	220/90 155/80	60.0	28.0	15.41	320	5020	0.27	3.93	11.03	1.5	28.8	Milk Diet
													Average—	2.2	28.7	

Normal Non-pregnant, 26.5
Normal Pregnancy, 16.4
Nephritic Toxemia, 22.8
Pre-eclamptic Toxemia, 26.2
True Eclampsia, 28.7

We are, as yet, not in a position to give definite values for the several types of toxemias, although our figures seem to indicate that X has its highest value in true eclampsia; while in nephritic toxemia the value is above that found in normal pregnancy; and in pre-eclamptic toxemia it is slightly higher than in the nephritic type.

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THE AFTER-EFFECTS OF THE LATE TOXEMIAS OF PREGNANCY

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In spite of the wealth of material in the literature dealing with the late toxemias of pregnancy, there is little to be found concerning their subsequent effects, particularly as regards the part they play in the production of permanent damage to the kidneys. Slemons [1] reported a series of eighteen private patients whose first pregnancy had been complicated by toxemia and who subsequently became pregnant. Fifteen were entirely free from toxemic symptoms in their subsequent pregnancies; while in the other three each pregnancy was complicated by toxemia. Of the latter group, two were twice pregnant and recovered; while the third died from nephritis several months after her fourth pregnancy had been terminated because of pronounced albuminuria. Le Page [2] studied a series of thirty-eight hospital patients whose pregnancies had been complicated by convulsions or by albuminuria severe enough to warrant interruption of pregnancy, and found that twenty-one of them had subsequent pregnancies complicated by toxemia.

During the past four years I have attempted to have every patient who was admitted to the service suffering from any variety of the late toxemias of pregnancy return for further study at the end of one year following delivery, in the hope of obtaining information concerning the ultimate outcome of such complications.

From February 1, 1919, to February 1, 1923, one hundred and seventy-seven such patients had two hundred and eighteen deliveries in the Obstetrical Department of the Johns Hopkins Hospital. Thirteen of them died in the service and one other died in another hospital six months after delivery, while one hundred and eleven of the surviving one hundred and sixty-three patients * returned for further study at the end of one year.

At the year return visit, I saw each patient personally, and a history was taken with particular reference to the existence of symptoms indicative of renal disease or of other illnesses which had occurred following discharge from the hospital. A careful physical examination was made, including the determination of the blood pressure and the usual routine examination of the urine. In addition, the eye grounds were examined by Dr. C. A. Clapp, of the Department of Ophthalmology, to whom I am greatly indebted for his constant interest and great assistance. It is realized that elaborate metabolic studies might have led to findings of great value, but in most

* That such a large number of return visits were obtained was due, in great part, to the efforts of Miss Esther Spicer, Chief Social Worker of the Obstetrical Department, to whom I desire to express my thanks for her coöperation, without which this study would have been impossible.

instances this was not possible. While it may be admitted that some of the mild cases of nephritis may have escaped recognition, it is not thought that we diagnosed the existence of renal involvement in any patients who were normal.

Classification of the Late Toxemias of Pregnancy

In studying the late toxemias of pregnancy one is impressed by the fact that there is little unanimity of opinion concerning their proper classification. Slemons [1] points out the danger of regarding all toxemias as being due to a common etiological factor or as representing a single pathological entity, and has directed attention to the necessity for careful differentiation between the various types, because upon such differentiation depends, in large measure, the fate of such patients in future pregnancies.

The classification which we have employed is based primarily upon two criteria; the presence or absence of convulsions and the presence or absence of renal involvement at the end of three weeks following the termination of pregnancy. In no case have we felt that a conclusive diagnosis can be made before the expiration of this period, save in patients who die and come to autopsy. Based upon the above criteria, the toxemias of our patients have been divided into four groups—eclampsia, preeclamptic toxemia, nephritic toxemia with convulsions, and nephritic toxemia without convulsions. This classification does not include the rare cases of eclampsia without convulsions, acute yellow atrophy of the liver, nor the so-called presumable toxemias, no example of which occurred in our series.

Before undertaking this investigation, it had been our belief that both eclampsia and preeclamptic toxemia were rarely followed by permanent damage to the kidneys, but actually seemed to confer a relative immunity against subsequent attacks, with the result that, in such patients, the condition was less likely to recur than in women whose previous pregnancies had been devoid of such complications. On the other hand, our experience had taught us that in women suffering from nephritic toxemia either with or without convulsions the complication invariably recurs in subsequent pregnancies in an increasingly severe form and manifests itself earlier in each successive pregnancy.

Eclampsia

As shown in Table I, in our series of 177 patients, the final diagnosis of eclampsia was made in fifty-one instances,—in thirty-eight primiparae and thirteen multiparae. Forty-two were delivered at term and nine prematurely, the earliest delivery in the series occurring at the twenty-eighth week of pregnancy. Nine of these patients died in the service and forty-two were discharged from the hospital entirely free from any sign of the disease—a mortality of 17.6 per cent. In each fatal case death was directly attributable to the eclampsia. Of the fifty-three babies—double ovum twins occurring twice in the series—twenty-two were discharged from the hospital in good condition, and thirty-one were stillborn or died during the first three weeks of life, a mortality of 58.4 per cent; although it should be remembered that seven of the children who died were premature.

Of the forty-two mothers discharged from the hospital entirely free from signs and symptoms of the disease, twenty-seven returned for study one year later, and in twenty-four of them we were unable to find any evidence of renal damage. Furthermore, it should be mentioned that eleven of these patients had additional deliveries in the service subsequent to the original eclamptic attack—nine having had one and two two labors each, none of which were associated with toxemic or renal complications. On the other hand, three patients who returned showed positive signs of chronic nephritis, and two of them, likewise, had subsequent labors, one having one and the other two deliveries in the service following the original eclampsia and, in all three pregnancies, there was persistence of the chronic nephritis which had been recognized at the year return visit. Consequently, our figures show that chronic nephritis developed in three out of twenty-seven women following an eclamptic attack, and in all probability resulted from it; since from the history and physical examination no other etiological factor could be elicited. While our figures are too small to justify figuring out the percentage incidence of chronic nephritis, they clearly indicate that it is a sequel to be reckoned with in women who have had eclampsia, but at the same time they apparently afford proof of the correctness of the belief that one attack of eclampsia confers at least a relative immunity against its occurrence in subsequent pregnancies. At least it did not occur in any of the 16 subsequent pregnancies in our series.

TABLE I.

Eclampsia.

	Died in service	Returned normal	Returned with nephritis	Total return visits	Failed to return	Total
Primiparae . . .	6	18	2	20	12	38
Multiparae . . .	3	6	1	7	3	13
Total.	9	24	3	27	15	51

Preeclamptic Toxemia

In our series the diagnosis of preeclamptic toxemia was made in eighty-three patients, fifty-six primiparae and twenty-seven multiparae, as shown in Table II. Seventy-one were delivered at term and twelve prematurely, the earliest delivery occurring in the twenty-eighth week of pregnancy. In none of the patients did the history or physical findings indicate the existence of chronic nephritis during the pregnancy or puerperium in question, and in the case of the multiparae, in so far as we could ascer-

tain, the previous pregnancies had been free from toxemic complications. On their discharge from the hospital all of these patients were free from symptoms of the disease as indicated by the absence of albuminuria and casts, as well as by a blood pressure within the normal limits. Eighty-five children were delivered—double ovum twins occurring twice—and seventy-seven of them were discharged from the hospital in good condition, while eight were stillborn or died during the first three weeks of life including five who were premature, a mortality of 9.4 per cent.

Fifty-five of the eighty-three patients returned for examination at the end of one year, and to our great surprise only twenty-two of them were normal, while in the remaining thirty-three signs of chronic renal involvement were present. Here, again, neither the history nor physical examination gave information as to the cause of the nephritis, so that we are forced to conclude that, in all probability, the previous toxemia must have been concerned in its etiology. When the fifty-five patients are studied according to the number of children which they have borne, the results are still more striking. Our figures show that thirty-four were primiparae and twenty-one multiparae, and that fifteen of the former and eighteen of the latter presented evidences of chronic renal disease—a percentage incidence of 44 and 86, respectively. In other words, while the incidence of chronic nephritis was unexpectedly high in both groups, it was twice as great in multiparae as in primiparae.

Nineteen subsequent pregnancies have occurred among the fifty-five patients in question, nine in seven patients who were normal at the year return visit, and ten in eight patients who presented signs of chronic nephritis at that time. In the first group no patient showed evidences of toxemia or renal deficiency in her subsequent pregnancies; while in the second group each of the ten pregnancies was complicated by nephritic toxemia, signs of which persisted as long as the patients were under observation.

Analysis of the findings in the preeclamptic group serves to demonstrate the incorrectness of the generally accepted view that preeclamptic toxemia rarely eventuates in chronic nephritis, inasmuch as 60 per cent of our patients in whom this diagnosis was made, and who returned at the end of the year for further study, presented indubitable evidence of its existence.

Such a result was unexpected and caused us the greatest surprise. In the first place, we are accustomed to regard preeclamptic toxemia as the precursor of eclampsia and to base its prophylaxis upon the early recognition and treatment of the former. Yet, we find in our patients that the sequelae of what we have hitherto regarded as the less serious condition are more important and occur much more frequently than after the major one. Moreover, the evidence at our disposal indicates that if the patient has an eclamptic attack and does not succumb to it, the ultimate outcome is more favorable than if she had a preeclamptic attack. Such a conclusion seems to be almost a contradiction of terms, and requires an explanation, which we are unfortunately not yet in a position to give.

Generally speaking, the likeliest explanation is that our means of diagnosticating preeclamptic toxemia are faulty, and that we group under that caption two conditions radically different but which as yet we are unable to differentiate. One is the actual forerunner of eclampsia, and leaves the patient in good condition if the eclamptic attack is averted or recovered from, while the second is really a larval form of chronic nephritis. Two sets of considerations may be adduced in support of such a contention—first, the well known uncertainty concerning the prognostic evaluation in a given case of preeclamptic toxemia, as every one can recall the unexpected outbreak of eclampsia in patients in whom the prophylactic treatment seemed to be progressing satisfactorily, and, on the other hand, the failure of eclampsia to occur in patients in whom it appeared imminent. Second, the results noted in our series of patients in whom pregnancy occurred subsequently to the year return examination following the original attack of toxemia. Thus, in the nine pregnancies occurring in the seven women who were normal at that time there was no sign of further trouble, while signs of chronic nephritis persisted throughout the ten pregnancies of the eight women who were not normal. In the circumstances, it seems permissible to surmise that the conditions underlying the two groups of cases must be radically different, but that they are as yet undistinguishable by our present means of diagnosis.

TABLE II.

Preeclamptic toxemia.

	Returned normal	Returned with nephritis	Total return visits	Failed to return	Total
Primiparae	19	15	34	22	56
Multiparae	3	18	21	6	27
Total	22	33	55	28	83

NEPHRITIC TOXEMIA WITH AND WITHOUT CONVULSIONS

As shown in Table III, our series of pregnancies complicated by nephritic toxemia is made up of forty-three patients, nine of whom had convulsions. Nine were primiparae, thirty-four were multiparae; nineteen were delivered at term and twenty-four prematurely, the earliest termination of pregnancy occurring at the tenth week. Four deaths occurred in the service; two as the result of uremic coma, one of uremic convulsions and one of hemorrhage resulting from premature separation of the normally implanted placenta. Thirty-nine patients were discharged from the hospital three weeks or more after delivery, all with evidences of chronic nephritis. Nineteen children were discharged from the hospital in good con-

dition, while twenty-four, twenty of which were premature, were stillborn or died during the first three weeks of life.

Of the thirty-nine patients who were discharged alive, one died in uremic coma in another hospital six months after delivery, and thirty returned for examination at the end of one year. In all patients, who returned, evidences of chronic nephritis were present, and, in fact, its manifestations were more severe than when the patient left the hospital. Six of these patients each had one subsequent delivery in the service and in each instance the second pregnancy was complicated by a nephritic toxemia more severe and making itself manifest earlier than in the previous one.

In summary, it suffices to say that our cases of nephritic toxemia bear out the generally accepted view that the underlying cause of the complication is a chronic nephritis and that the complication makes itself manifest in increasing severity and at an earlier period in each succeeding pregnancy.

TABLE III.

Nephritic toxemia with and without convulsions.

	Died in service	Died elsewhere	Returned normal	Returned with nephritis	Failed to return	Total
Primiparae . . .	0	0	0	6	3	9
Multiparae . . .	4	1	0	24	5	34
Total	4	1	0	30	8	43

Duration of Symptoms

Our studies upon the after-effects of eclampsia and, more particularly, of preeclamptic toxemia show that the absence of signs of nephritis three weeks after delivery in no wise precludes the possibility of permanent renal damage. This is rather to be expected when it is remembered that, owing to the restriction of activity and diet in the hospital, the evidence of nephritis may well be absent in mild cases. In searching for a means of differentiating between the cases of toxemia which will be followed by permanent renal injury and those in which no such sequelae occur, it seemed possible that an inquiry into the duration of the toxemia before delivery might throw light upon the question as to whether the kidneys were to escape permanent injury or not. Accordingly, our patients were studied from this point of view and in every instance an attempt was made to obtain an accurate history as to how long symptoms of the toxemia had persisted before delivery.

Thus, among the fifty-one patients who had eclampsia the most usual duration of premonitory symptoms was less than one week. In ten patients they were entirely lacking, while in eight they had persisted for more than four weeks. It is interesting to note that in the three patients who were found to have chronic nephritis one year following eclampsia the premonitory symptoms had preceded the eclamptic outbreak by one month in one patient and by two months in the other two.

On the other hand, in the group of patients showing preeclamptic toxemia the most frequent duration of symptoms before delivery was more than four weeks, forty of our eighty-three patients belonging in this group. To particularize, in the patients who were normal at the year return visit symptoms of preeclamptic toxemia had preceded delivery for more than four weeks in thirty-six per cent, as compared with sixty-five per cent in those who presented chronic nephritis one year following delivery. Furthermore, in the forty-three patients whose pregnancies were complicated by nephritic toxemia the symptoms had preceded delivery for more than four weeks in thirty-eight, an incidence of 88 per cent.

The Blood Pressure and Albumin During the First Week After Delivery

It is generally held that the blood pressure falls and the albumin decreases much more rapidly during the first week or ten days after delivery when the complicating toxemia is of the eclamptic or preeclamptic type than when it is nephritic in origin. However, this does not help us in attempting to differentiate between those cases of preeclamptic toxemia which will be followed by chronic nephritis and those which will show no signs of permanent renal damage. For example, in twenty of our thirty-three patients who returned at the end of one year with signs of nephritis, the blood pressure had fallen to within normal limits and the albumin had decreased to a trace within the week following delivery. On the other hand, in nine of our twenty-two patients who were normal at the year return visit the blood pressure remained elevated and more than a trace of albumin was present one week after delivery.

Discussion

Until we know the causes of the late toxemias of pregnancy, we must content ourselves with speculations concerning the various factors concerned in this causation and the effect of such factors. However, our findings clearly indicate that the late effects of eclampsia and, more particularly, of preeclamptic toxemia are more severe than are generally supposed. Our four years' study of all patients presenting the late toxemias of pregnancy in our service shows that we are in no wise justified in assuring those who have suffered from eclampsia or preeclamptic toxemia that they may face future pregnancies without fear of toxemic complications.

Furthermore, the length of time the toxemic symptoms have persisted seems to be a factor in determining the occurrence of permanent renal damage. This is of special interest in the management of preeclamptic toxemia, as many patients with this complication are brought into the hospital, kept at rest, placed upon a restricted diet

and subjected to methods supposed to promote elimination. If urgent symptoms do not arise, the treatment in many instances is persisted in until term is reached and labor occurs spontaneously. That such a procedure results in a very low fetal mortality our figures amply prove, but in view of our experience it seems pertinent to inquire whether we may not seriously increase the chances of permanent renal injury by allowing the pregnancy to continue too long? We are not in a position to answer this question with assurance, but our experience leads us to think that such may be the case.

Whether preeclamptic toxemia is a disease entity which, when it runs a prolonged course, produces permanent damage to the kidneys, or whether it is itself really a manifestation of chronic nephritis produced by the added strain on the kidneys as a result of pregnancy, is another question, the answer to which must await the future and the discussion of which, with our present knowledge, can only lead to speculation of the most inconclusive kind. In this connection, the fact that preeclamptic toxemia is much more frequently followed by chronic nephritis in multiparae than in primiparae leads one to wonder whether pregnancy does not subject the kidneys to a greater burden than is generally supposed. We do not know, and all that we can say at present is that our experience indicates that permanent renal damage is much more likely to follow preeclamptic toxemia than eclampsia, and it is suggestive that the former usually runs a much more prolonged course.

Although we have found that the disappearance of the signs and symptoms of the toxemia within three weeks after delivery does not in any way preclude the possibility of permanent renal injury, it is nevertheless true that, when these persist for a longer period, evidences of chronic nephritis were found in every patient who was seen one year later. Consequently, our experience seems to justify the conclusion that, when the signs and symptoms of the toxemia persist for three weeks or longer after delivery, the chances of escaping permanently damaged kidneys are minimal.

In those patients who were discharged at the end of three weeks free from the signs and symptoms of the toxemia, and yet in whom, at the year return visit, evidences of chronic nephritis were found, the question naturally arises as to when such evidences first became manifest. We cannot answer this question because, unfortunately, we were not able to follow our patients during the interval between their discharge from the hospital and their return at the end of the year. This is a point of practical importance, for the findings made at that visit were borne out by the behavior in the subsequent pregnancies, with the result that those who were normal at that time remained so, while those who presented signs of chronic nephritis continued to manifest it during subsequent pregnancies.

Our experience with nephritic toxemia with and without convulsions has added nothing to our knowledge of the subject, except to confirm the view that the prognosis becomes increasingly graver in each succeeding pregnancy.

CONCLUSIONS

Although our series is too small to answer many of the questions which we have raised, the following conclusions seem justified.

1. While no recurrences of eclampsia occurred in our series, the fact that three of the twenty-seven eclamptic patients who were seen one year later showed evidences of chronic nephritis, indicates that the danger of permanent renal damage following eclampsia is not to be disregarded.

2. The danger of chronic nephritis following preeclamptic toxemia is unexpectedly great, as shown by the fact that 60 per cent of our patients whose pregnancies were complicated by preeclamptic toxemia showed evidences of chronic renal disease when examined one year later.

3. We are unable to differentiate between the cases of preeclamptic toxemia which will be followed by chronic nephritis and those which will not result in permanent renal injury. It is possible that the duration of the symptoms of the toxemia before delivery may be an important factor.

4. If, in supposed cases of preeclamptic toxemia, the evidences of the toxemia persist for three weeks or more after delivery, the presumption is that the underlying disease is renal in origin.

It is planned to continue this investigation, supplementing it with more elaborate metabolic tests, especially with chemical studies of the blood and urine.

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GLIOMATOSIS OF THE LEPTOMENINGES

By W. M. Firor and F. R. Ford

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Nine cases of diffuse gliomatous invasion of the pia-arachnoid have been reported up to the present time. The first was described by Pels-Leusden in 1898.[1] This tumor was composed of small round cells with a fibrous stroma which gave the staining reactions of glial tissue. He termed the growth a "glio-sarcoma." In the same year two similar cases were published; one by Fränkel [2] and one by Roux and Paviot.[3] Since then Fischer,[4] Grund,[5] Spiller,[6] Martens and Seiffert,[7] Verdun,[8] and Ford and Firor [10] have all reported cases of this same condition.

Before 1898 pathological literature contains no mention of metastasizing gliomas; yet the so-called primary sarcomatosis of the meninges was already well known. This latter condition had been described as early as 1837 by Ollivier.[13] Since the report of Pels-Leusden's case it has become increasingly evident that these two groups of cases are very closely related, and that some cases formerly classified as sarcomas are in reality of glial origin. In some of these cases glial fibers may be demonstrated by special stains. In others no glial tissue can be found; and yet, Spiller,[6] Grund,[5] Borst,[15] and Ewing [17] believe that this fact alone does not disprove their possible glial origin, and that gliomas may consist entirely of cells. Verdun [8,9] has claimed that these growths originate in proliferations of the subependymal glial tissue which rupture through the ependyma and metastasize by way of the cerebro-spinal fluid, and Martens and Seiffert were led to take the same point of view from the study of their case. Ependymomas, which may be regarded as developing from a special type of glial tissue, also metastasize to the meninges.[11,12] Local gliomatous invasion of the meninges has been observed in the region of a cerebral glioma which involves the surface of the cortex.[14,15] This article is based on one case not previously reported and abstracts of the nine cases mentioned above.

*Case I** *Summary*: boy of six with pain in the back and paralysis and atrophy of left leg of one year's duration, hydrocephalus, "convolutional atrophy" of skull, and enlarged sella turcica, yellow spinal fluid. Operation and removal of mass of tumor tissue compressing lumbar cord; death. Autopsy: Metastatic infiltration of cerebral and spinal meninges from cystic glioma in region of infundibulum, internal hydrocephalus.

W. W., aged six years, was admitted to Johns Hopkins Hospital on November 15, 1922, complaining of paralysis of the left leg. One year previously a severe pain had developed in the back and in the left leg. Several months later his left leg had become weak and his back tender so that he could not lie on it. Several months before entrance to the hospital, he had been confined to bed and suffered from incontinence of urine. There was no history of headache or vomiting.

In the hospital the patient always lay on his left side with his legs flexed at the hip and knee. His spine was flexed and there was a smooth round kyphos in the lumbar region. His head was definitely hydrocephalic. The optic fundi showed no pathological changes and the cranial nerves were normal. There was a flaccid paralysis of the calf muscles on the left with some atrophy and a partial reaction of degeneration. The right leg could be moved freely. There was complete loss of sphincter control. Over the left leg up to the hip there seemed to be a slight loss of all forms of cutaneous sensibility. There was tenderness on pressure over all the lumbar vertebrae and any attempt to extend the spine caused severe pain. The right knee jerk was feeble; the left could not be obtained. Both ankle jerks were increased and there was a bilateral ankle clonus. Plantar stimulation gave no response. Lumbar puncture yielded yellow fluid which contained a few red blood cells and some white cells. No search for atypical cells was made. The Wassermann test was always negative in the blood and spinal fluid. An x-ray examination of the skull showed a greatly enlarged pituitary fossa and extreme "convolutional atrophy" of the cranial bones.

On December 27, an intraspinal injection of air was made. The air did not reach the cranium, but stopped sharply at the level of the first lumbar vertebra. The following day Dr. Dandy operated and removed a tumor about 3 cm. in length which had compressed the left side of the conus medullaris. The patient showed no improvement, however, and died on January 9, 1923.

At the autopsy, which was performed by Dr. Margaret G. Smith, the convolutions over the convexity were seen to be flattened and the sulci obliterated, but the pia-arachnoid looked normal. In the region of the infundibulum there was a cystic mass of soft reddish tissue which involved the chiasm and the optic nerves, and extended posteriorly in a thin layer over the entire base of the brain to the medulla, completely filling the arachnoidal cisternae. The cranial nerves were embedded in the mass. All the ventricles were dilated and in places the cortex was reduced to a thickness of 2 cm. There was no obstruction in the ventricular system, and the foramina of the fourth

* By the courtesy of Dr. Walter E. Dandy.

ventricle were patent. A solution of India ink which had been injected intra-spinously before autopsy could be found in the ventricles, but not under the base or over the convexity. The medulla and the posterior part of the pons were colored black by ink. The cord was completely ensheathed by a thin layer of tumor tissue and all the spinal nerve roots were encrusted with the growth. The conus medullaris was softened and the left half replaced by blood clots and shreds of tumor tissue. This was evidently the location of the tumor removed at operation.

Microscopically, the tumor was composed of small cells with oblong nuclei embedded in a delicate, loose, reticular stroma. Scattered through this tissue were many larger cells, round or polygonal in shape, with one or two large pale nuclei which showed heavy masses of chromatin and distinct nucleoli. Unmistakable glial fibers were demonstrated by the phosphotungstic acid stain (See Figs. 1 and 2). The tumor cells were found scattered throughout the subarachnoid space and infiltrating the pia and arachnoid. In the region of the conus medullaris, at the site of operation, there was quite definite invasion of the cord.

Case II (Pels-Leusden).[1] Man of 22 with pain and weakness of back and legs, headache and loss of vision. Examination: vision on left good, on right reduced one half, bilateral papilledema, convergent strabismus, paralysis of legs and anesthesia over legs and sacral segments, retention of urine, loss of knee jerks; later, clonic spasms of legs, stiff neck, nystagmus, delirium, coma; death over a year after onset. Autopsy: brain soft, convolutions flattened, pia at base infiltrated especially about chiasm, pons and peduncles also ensheathed by tumor tissue, marked hydrocephalus, tumor masses in left lateral ventricle, cord surrounded by growth and compressed and softened in lumbar region by tumor mass, brown fluid in spinal subarachnoid space; microscopically, round cells with glial fibers; tumor termed "glio-sarcoma."

Case III (Fränkel).[2] Man of 33 with pain in neck, shoulders and back, and weakness in all four extremities of one month's duration. Examination: marked atrophy of shoulder girdle and arm muscles, flaccid weakness of arms and spastic weakness of legs, gait unsteady, tendon reflexes in legs increased, bilateral ankle clonus, loss of abdominal reflexes, some slight loss of cutaneous sensibility on hands and feet, no cranial symptoms, fundi normal, no headache; later, complete paralysis of extremities, retention of urine, loss of pain sensibility on arms and legs, loss of all reflexes, general hyperesthesia, inequality of pupils; bedsores and death 2 months after onset. Autopsy; pia at base thickened, medulla and cord surrounded by thick layer of tumor tissue, cord compressed and softened in upper cervical region by large tumor mass in pia, tumor nodules on posterior nerve roots and cauda equina, tumor nodule in center of cord at about T 5; microscopically, extensive degenerations in cord, tumor composed of small round cells with few glial fibers; termed "glio-sarcoma."

Case IV (Roux et Paviot).[3] Man of 45 with pain in left arm, back and legs, and numbness of soles of feet. Examination: cranial nerves normal, legs spastic and weak, arms slightly paretic, knee jerks increased, bilateral ankle clonus, difficulty in starting urine; later, complete paralysis and anesthesia below C 6; death from pneumonia 7 months after onset. Autopsy: large tumor mass invading cord, extending from C 5 to T 2, spinal pia-arachnoid infiltrated and cauda equina and spinal roots thickened and nodular, no note about brain or cerebral meninges; microscopically, glioma.

Case V (Fischer).[4] Girl of 8 with pain in abdomen and back and weakness of legs. Examination: complete flaccid paraplegia, loss of sensibility below T 5 on right, and below T 7 on left, loss of sphincter control; later, weakness and finally paralysis of arms, inequality of pupils, and bilateral papilledema, death 8 months after onset. Autopsy: tumor masses in base of third ventricle and in head of caudate nucleus; medulla, base of fourth ventricle and corpora quadrigemina all infiltrated, lumbar cord completely destroyed by growth which had eroded into abdominal cavity, central cavitation in cervical cord; composed of small round cells and some larger multinuclear cells with glial fibers; classified as a "glio-sarcoma."

Case VI (Grund).[5] Boy of 11 with weakness of right arm of 2 months' duration and headaches and nausea of 2 weeks' duration. Examination: pupils dilated, right larger than left, other cranial nerves normal, whole right arm and shoulder weak and atrophic, slight subjective sensory loss over right arm, brachial nerve trunks tender on right, constant pain in both shoulders, knee jerks increased, plantar reflexes equivocal; later, headache and vomiting, beginning papilledema, progressive weakness of left arm, complete paralysis of right seventh nerve, nystagmus, deviation of tongue to right, paralysis of right sixth nerve, general hyperesthesia, weakness of right leg, bilateral facial paralysis, convergent strabismus, loss of knee jerks, spinal fluid yellow, pressure increased, no cells; coma and death 4 months after onset. Autopsy: pia-arachnoid thickened at base, especially about optic nerves, small nodules in Sylvian fissure, and in cerebral sulci, few minute nodules in pia over convexity, dilatation of all ventricles, few small nodules in choroid plexus of fourth ventricle, spinal pia-arachnoid thickly infiltrated throughout, roots of cauda equina surrounded by tumor tissue, large intra-medullary tumor in cervical region extending inside cord from C 3 to C 8 continuous with extra-medullary growth at C 6, dura penetrated at C 6, no statement about presence or absence of growth in lateral ventricles; microscopically, small round and spindle cells with fine reticular stroma which gives all the staining reactions of glial tissue, perivascular lymph-spaces full of tumor cells.

Case VII (Spiller).[6] Man of 45 with headache vomiting and vertigo; failing vision for one year, sudden loss of consciousness twice, without convulsion. Examination: bilateral papilledema, vision one half on right side, reduced to counting fingers on left, pupils unequal, left greater than right, bilateral sixth nerve paralysis, total loss of hearing in right ear, very deaf in left ear, frequent spells of intense vertigo with forced movements, no weakness of fifth or seventh nerves detected, no weakness of extremities, finger nose test shows slight tremor, gait unsteady and ataxic, knee jerks active and equal, death after cerebellar exploration. Autopsy: many small masses of tumor tissue about cranial nerves in each cerebellopontine angle, and in subarachnoid space at base; optic chiasm, infundibulum, olfactory bulbs, and Gasserian ganglia all surrounded by tumor tissure, each internal auditory foramen full of tumor growth, large mass in fourth ventricle infiltrating posterior part of medulla, spinal pia thickened and nodular, posterior nerve roots and cauda equina show minute nodules, diffuse infiltration of pia over posterior surface of cord; microscopically, left optic nerve sheath full of tumor cells, fresh degenerations by Marchi method in all cranial nerves including seventh, degenerated fibers in posterior columns of cord, tumor tissue composed of small round cells with no fibers, in places columnar cells arranged in alveoli as in ependymomas; diagnosis, glioma.

Case VIII (Martens and Seiffert).[7] Boy of 13 with headache and diplopia. Examination: bilateral exophthalmos, bilateral papilledema, slight weakness of left side of face, nystagmoid movements of eyes, walks to right, station unsteady, diplopia in right lateral fields, stiff neck, death after cerebellar exploration. Autopsy: pia over cerebellar hemispheres thickened, tumor mass in roof of fourth ventricle and many small masses scattered over ependyma of third and lateral ventricles, moderate hydrocephalus, no note about meninges at base or over cord; microscopically, small round cells with a few glial fibers; classified as subependymal glioma.

Case IX (Verdun).[8,9] Man of 21 with headache and vomiting of 6 weeks' duration. Examination: stiffness of neck, positive Kernig, general hyperesthesia, diminished tendon reflexes, weakness of right sixth nerve, bilateral papilledema, amblyopia, general weakness of extremities, no paralysis, atypical cells in spinal fluid; delirium and death 3 months after onset. Autopsy: infiltration of meninges at base and about chiasm, large tumor in corpus callosum and posterior horn of right lateral ventricle, pin head tumors on ependymal surface, spinal meninges infiltrated with tumor cells; microscopically, cellular tissue with fine glial fibers, composed of small round cells and large multinuclear cells; classified as subependymal glioma.

Case X (Ford and Firor).[10] * Boy of 15 months with vomiting, strabismus, bilateral facial paralysis and unsteady gait. Examination: optic fundi normal, paralysis of left third nerve and both seventh nerves, apparently deaf, slight stiff neck, and positive Kernig; later, corneal ulcers and paralysis of jaws, inability to swallow; finally, paralysis of all cranial nerves, no paralysis of extremities, or modification of reflexes, bloody spinal fluid with large atypical cells; death 3 months after onset. Autopsy: large tumor in fourth ventricle, thick layer of tumor tissue under base from chiasm to pons, cranial nerves infiltrated and softened, moderate internal hydrocephalus, "granular ependymitis," spinal meninges thickened and infiltrated, cord normal; microscopically, small spindle cells in a delicate stroma containing glial fibers (see Fig. 3).

Clinical Discussion

The clinical picture which these cases present is variable and yet frequently characteristic. Cases I, II, V, and VI showed not only the signs of increased intracranial pressure, but a transverse lesion of the spinal cord as well. In Cases III and IV nothing more than the usual signs of a spinal cord tumor could be made out; and conversely, Cases VII, VIII, IX and X showed only symptoms referable to the cranium without any signs of cord involvement. Papilledema was present in six cases. In two cases the optic fundi were normal, although there was undoubtedly increased intra-cranial pressure. Cranial nerve palsies, apart from sixth nerve palsies which are often caused by increased intra-cranial pressure alone, were found in only four cases. In Case VII there was bilateral involvement of both eighth nerves, a condition which occurs not infrequently in "sarcomatosis" of the meninges. In Case X all the cranial nerves were paralyzed before death. "Meningeal" symptoms were emphasized in Case IX. In no case was there any localizing sign of an intra-cerebral growth, such as hemiplegia, aphasia, or hemianopia. The age of the patients varied from fifteen months to forty-five years. The average course was about seven months.

The diagnosis was made before death only in Verdun's case, in which atypical cells were found in the spinal fluid. A few of these cells were large and multinuclear; others were small and round. Verdun believed that some of these cells were tumor cells. Atypical cells were also found in the spinal fluid from Case X. In three cases the spinal fluid was yellow, but no atypical cells were noted.

Pathology

Histologically, these tumors are very cellular, and except in the first case, are quite unlike the usual type of

* By the courtesy of Dr. John Howland from the Harriet Lane Home for Children.

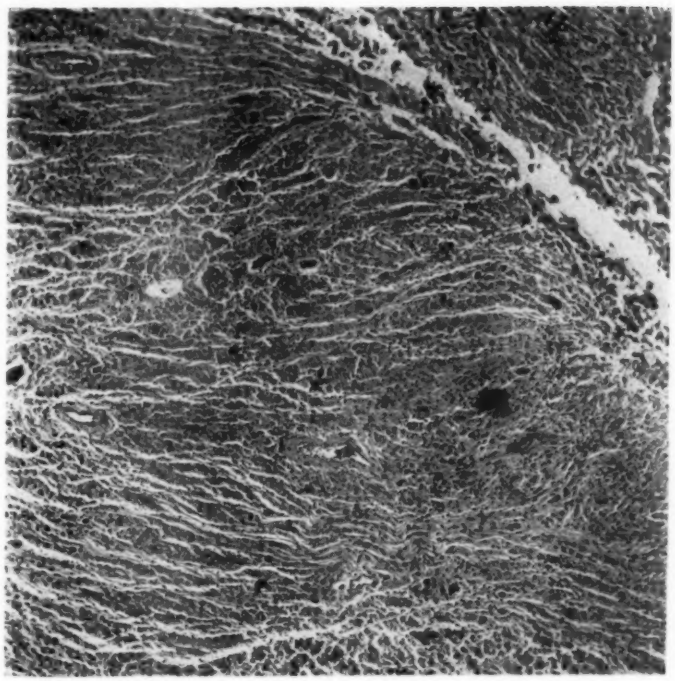

Fig. 1.—Low power microphotograph of tumor nodule in spinal canal. Case I.

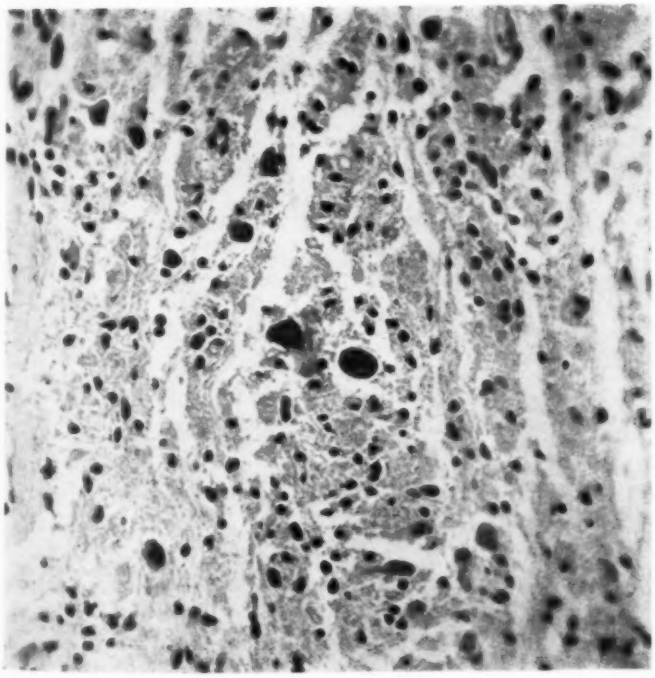

Fig. 2.—High power microphotograph of tumor tissue showing small cells and large multinuclear cells with glial fibers Case I. Same section as shown in Fig. 1.

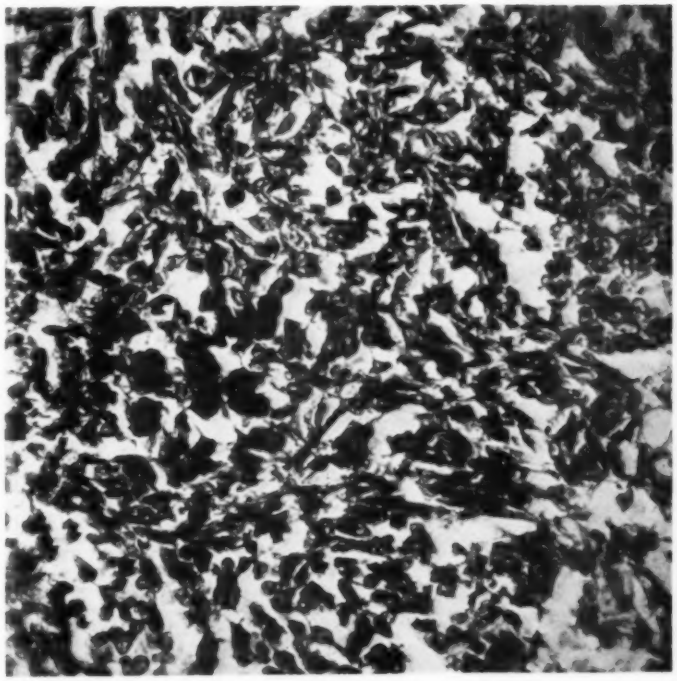

Fig. 3.—Microphotograph of tumor mass in cerebello-pontine angle to show short glial fibers. Case X.

glioma. They are composed of small round cells with a fine reticular stroma which gives the staining reactions of glial tissue. There were also some large multinuclear cells found in the cases of Fischer and Verdun. In Spiller's case alone there were no glial fibers to be found. In six cases the primary growth was in the cerebral ventricles; in three cases in the spinal cord, and in one case the growth apparently arose in the region of the infundibulum. In seven cases the meninges under the base of the brain as well as the spinal meninges were infiltrated. In three cases the tumor was confined to the spinal canal. The pia-arachnoid over the convexity was not affected. The cranial nerves and spinal roots were frequently infiltrated by tumor cells, and in Spiller's case there was some degeneration demonstrable by the Marchi stain in the cranial nerves spinal nerve roots and posterior columns of the cord.

SUMMARY

1. A case of gliomatosis of the pia-arachnoid is reported and nine cases are abstracted from the literature. The clinical and pathological characteristics of these cases are briefly analyzed.

2. The clinical picture is composed in varying proportions of signs of increased intra-cranial pressure, signs of spinal cord lesions, cranial nerve palsies and, rarely, of signs of meningeal irritation.

3. Pathologically, these tumors are very cellular gliomas with few fibers. They usually originate in the subependymal glial tissue or in the spinal cord, and secondarily involve the leptomeninges.

4. The diagnosis is difficult, but may be made by the demonstration of "tumor" cells in the spinal fluid.

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THE EFFECTS OF CHRONIC IRRITATIONS ON THE MORPHOLOGY OF THE PERITONEAL MESOTHELIUM

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There are two principal questions involved in the study of the lining cells of the peritoneal cavity under the influence of irritations. The first of these involves the specificity of the serosal cell under these changed conditions. Many observers claim that the serosal cell becomes changed to a typical phagocytic cell when free in the exudate, thus assuming characteristics and performing functions entirely foreign to the serosal cell under normal conditions. Other workers consider the serosal cell as being interchangeable with the fibroblast after any irritation of the peritoneum; the fibroblast being described as forming a new layer to cover surfaces denuded of serosal cells, while the latter are supposed to assist in the formation of new connective tissue. This question, involving the relationship of the serosal cells to the fibroblasts and to the active phagocytic cells of peritoneal exudates, I have discussed elsewhere,[3,5] and have pointed out the essential dissimilarity between these groups of cells. I have noted, however, the fact that the serosal cell is much more closely allied to the fibroblast in its reactions than to the phagocytic types of cells.

The second problem, which is of interest in any study of the peritoneum, deals with the changes produced by irritations in their bearing upon the formation of adhesions. In the literature concerning this phase of the problem there is much evidence that injury to the serosal cells is followed by the formation of adhesions, and these observations have caused surgeons to develop a rigorously care-

ful technique in regard to the peritoneal membranes during abdominal operations. But, on the other hand, very little has been done to examine carefully by histological methods the course and progress of the changes which follow injury or inflammation, and in general, much of the work, in which there has been histological control, may be criticized because the methods employed to produce the irritation have caused such acute inflammation that too profound and rapid changes were produced. In such studies as those of Heinz,[7] in which turpentine was introduced into the peritoneal cavity, the destruction of the serosal cells was widespread in a very short period of time and there was a massive formation of adhesions.

Von Brunn [1] has published very interesting drawings of the serosal cells which have been irritated to a considerable degree, and he shows them as cells varying from cuboidal to tall columnar cells which have small processes or pseudo-cilia. Many observers have found such processes to be characteristic of these cells after inflammatory changes.

The essential fact that is involved in the results to be communicated here is that the unbroken continuous layer of mesothelial cells is essential to prevent the formation of adhesions; and that very profound alterations may be brought about in these cells without the development of adhesions, so long as the layer of surface cells is intact. Some of the more important stages in the development of these changes will be described and figured.

In studying the absorption of substances from the peritoneal cavity I have shown that changes in the serosal cells can be produced by repeated injections of solutions of dextrose.[6] Animals used in these experiments showed changes which were somewhat similar to those described by von Brunn but much less severe. I have since found that even physiological saline given in repeated injections will produce the earlier phases of these changes. In the course of many experiments with the serosal cells it has also become apparent that most remarkable transformations can be brought about as the result of long continued, low grade irritations, and in spite of such transformations the peritoneal serosal lining can still function in such a manner that no adhesions result, and the normal physiological life of the animal is not interfered with. These changes always pass through the stages that von Brunn has demonstrated, but may be carried very much further if the proper precautions are observed. Furthermore, there are many characteristic effects which may be obtained by the use of vital dyes which still further confirm the conclusions stated elsewhere [5] that the serosal lining cell is a very specific entity differentiated especially as a lining cell and having certain characteristics not possessed by fibroblasts or other connective-tissue cells.

Both cats and rabbits were used for these experiments; they differ in several minor particulars both in regard to their reaction to vital dyes and in the changes which are produced by irritations. But the essential facts are quite similar in demonstrating the especial fitness of the lining cells for the prevention of adhesions. The cat was found to be more resistant than the rabbit to the general effects of long continued exhibitions of blood or other irritants, used to produce changes in the peritoneal cavity; and, therefore, the maximal changes obtained were in cats.

It has been shown [4] that the normal mesothelial lining cell of the rabbit has a very specific reaction to vital dyes and that this is of widespread similarity, though cells from different areas react differently in minor degrees. Similar variations have also been noted in the cat, the most noteworthy being that, especially in the case of the spleen, the first small group of vital dye granules which accumulate are almost always in the basal zone of the cell and not in the peripheral part as is seen in similar cells in the rabbit (See Figs. 4, 19, 20, 21).

The methods have been in general those already reported; [6] the injection of sterile whole or laked blood, made isotonic by the addition of sodium chloride. In some experiments trypan blue or a solution of carmine was given instead of, or combined with, the blood. No effort was made to use matched blood for these experiments, as maximal effects were desired, but it would be of interest to transfuse whole blood from one of a matched pair into the peritoneal cavity of the other. And while it is most probable that matched blood would produce an entirely similar histological change, the experiment should be carried out, especially in view of the suggestions which are to be found in the literature that blood transfusions may be made in such a manner. While the changes observed in any one animal may, and usually do, vary considerably in different areas, a given amount of irritation usually produces about the same grade of change. In general the earliest change noted has been an increase in the size and number of the serosal nuclei, so that they stand out from the surfaces of the organs while the optical ends of the cells remain quite flattened. In some areas, especially the diaphragm and omentum, this stage is followed by a definite rounding up of the entire cell with a peripheral separation such as is shown in Figs. 10, 13 and 16.

Very early in any irritation of the mesothelium there is a peculiar change in the surfaces of the serosal cells—the development of small, fine projections which simulate cilia or a striated border (see Fig. 17). These have been often described;—Hertzler,[8] Cunningham,[6] von Brunn.[1] Hertzler suggests that they are due to the pulling apart of two peritoneal surfaces which have been stuck together. This explanation is insufficient, however, since they may appear even when there is a considerable ascites. Their significance is quite uncertain, but they are probably surface tension phenomena analogous to

the production of similar structures on free cells in peritoneal exudates.

Fig. 17 is a section from the spleen of a supposedly normal rabbit in whose peritoneal cavity a considerable amount of clear fluid was found at autopsy. Careful study of the spleen revealed several infarcts which extended to the surface. This section is from the surface just adjacent to such an infarct and its appearance indicates a mild irritation of some kind. The pseudo-cilia are not as long as they are at times, but are quite characteristic. Here, also, the change in the cells is quite similar to that usually found on the diaphragm, in that the inter-nuclear portions of the cells have not increased very markedly in size. On the diaphragm and omentum the cells round up to an almost completely spherical state, usually remaining in very close contact except where desquamation takes place. During proliferation the cells divide quite irregularly, but in such a manner that they spread out more and more to cover denuded areas. This type of division is shown in Fig. 13 (which is from the omentum) where the cells are attempting to spread out as desquamation occurs. The only case in which the diaphragm and omentum tend to assume the characteristics of the parenchymatous surfaces is when masses of exudate become adherent and these are in turn covered by the actively proliferating serosal cells. This is shown in Fig. 23 and will be referred to later.

In the case of the spleen and liver, which are typical of the parenchymatous organs, the earlier changes are quite similar to those in the omentum and diaphragm, while those developing later are very much more extensive. Here there is a rounding up of the cells and an increase in size of the nuclei; but very quickly the cell-bodies also increase in thickness and become first a layer of low cuboidal cells and later of true cuboidal, though of course always irregular, cells. These two stages are shown in Figs. 9, 1 and 3. In Fig. 3 the cells may be seen to be somewhat rounded on the surface, which is to be expected, since any such change tends to separate the cells to some extent. From this cuboidal stage the cells pass quickly to a columnar state, which varies considerably in different animals and with the extent of the irritation. Various modifications of this can be seen in Figs. 4, 6, 11, 12, 14 and 15. Fig. 4 shows the typical columnar form of the cells of the cat's spleen in which the cells are quite closely placed side by side with only moderate irregularity of the surfaces. On careful examination of this figure it will be seen that the base of each cell contains a clear area and in this clear area are a few blue granules (which can only be seen as fine black dots in the photograph). This section is from an animal that had received intravenous injections of trypan blue prior to, and during, the time that the irritation was being carried out. This storage of vital stain in the modified mesothelium of the spleen is quite similar to that in the germinal epithelium of the ovary which has been figured in another place.[4] The ovary from which the drawing just referred to was made has been photographed and is shown in Fig. 2. In the basal vacuoles of the germinal epithelium a few fine, grayish dots (the trypan blue aggregations) can be made out. This peculiar staining of the mesothelium of the cat's spleen is quite strikingly different from that seen in the rabbit where the dye tends usually to collect peripherally to the nucleus in a small clump, as shown in Figs. 19, 20 and 21. This peculiarity is probably of little functional significance although the observations noted elsewhere on the cat [2] must be extended to the rabbit in order to test this question and determine whether the polarity of the cells is functionally indicated by this vital staining.

Fig. 15 shows the maximal stage obtained in regard to the columnar type of proliferation. Here the cells are smoothly packed together just as in Fig. 4, but they have become much more elongated. In passing from such a stage as that shown in Figs. 3 and 4 to the stages shown in Figs. 5 and 6, the cells have become irregular. These irregularities are produced by the proliferation and desquamation of some cells, and by the overgrowth of others. In this process certain cells become pressed towards the surface and others remain more closely adherent to the underlying connective-tissue structure. These remaining serosal cells can again divide and form a new layer in case of too widespread desquamation of the more superficial cells. In Fig. 12, a serosal cell that has just divided in a perpendicular plane may be seen. Figs. 11 and 14 both show several cells that remain closely associated with the underlying structure but still are quite definitely not a part of it. Further stages can be seen in Figs. 5 and 6, the former showing the maximal change that has been produced in the serosal cells without the development of adhesions. In Fig. 6 can be seen the peripheral separation which takes place in these cells when the growth is very rapid and also a very sharp differentiation between the true serosal layer and the subjacent connective-tissue capsule. This point is also shown in Fig. 5 which, however, represents a longer irritation and a more advanced stage of mesothelial overgrowth. The cells which in Fig. 6 are seen to be definitely basal, i. e., left behind in the overgrowth, have begun to proliferate and the mass of cells is thicker, more closely packed together, and more regular on the surface.

Finally, when the irritation has been carried to a maximal state, there begin to be produced such changes as are shown in Figs. 7 and 8. Here the subjacent connective tissues have begun to proliferate and push out tufts of blood vessels and stroma, but the serosal lining, of greatly changed cells, proliferates very rapidly and covers these outgrowths so that the continuity of the serosal cells over the entire peritoneal surface is maintained. Thus it seems that, so long as the serosal cells remain intact, even a stimulus sufficient to produce such a fibroblastic change

as that shown in Fig. 8 is still not intense enough to cause the formation of adhesions.

Fig. 22 is from the diaphragm of a rabbit which has undergone considerable irritation from repeated injections of blood and has at the same time been stained vitally with trypan blue. A considerable amount of exudative material has collected on the surface and is in process of being covered by new mesothelial cells. These rounded-up serosal cells can be seen covering the external surface of the layer of exudate and also forming a lining to the cavity formed between this exudate and the original surface of the diaphragm. The point at which the serosal cells pass around the junction of the exudate and the surface of the diaphragm is shown in Fig. 24, more highly magnified. It can be seen that, while the layer of the serosal cells is irregular, it is obviously continuous and is everywhere quite similar. In Fig. 23 another area of this same mass of exudate is shown; here the original layer of serosal cells may be seen as a continuous layer of cuboidal cells of about the same degree of modification as that shown in Fig. 9; but the serosal cells have also formed a second layer external to the mass of exudate which consists of injected red blood cells, desquamated serosal cells, phagocytic mononuclears and other debris. There is, however, no outgrowth of blood vessels and connective-tissue stroma.

In regard to the serosal surfaces of the body-wall and intestine, the changes are usually much less extensive than those of the liver and spleen. Fig. 25 shows the maximal state of change so far induced in the cells of the body wall without the formation of adhesions.

Finally, the figures show in several places the enormous difference in the amount of vital dye that the phagocytic mononuclear cells of the tissues and the irritated serosal cells have in their cytoplasm. In Fig. 16 a large clasmatocyte loaded with trypan blue is seen just beneath the central rounded-up serosal cell. The serosal cells contain a few aggregations of the dye which show as small grayish dots in the cytoplasm. Figs. 23 and 24 also show clasmatocytes loaded with blue in the exudate and in the connective tissues.

Summary

It has been found that the injection of laked or whole blood, or any other mildly irritating substance, into the peritoneal cavity causes the serosal cells to increase in number and at the same time to round up, becoming at first cuboidal and finally columnar. When desquamation occurs, the remaining serosal cells proliferate rapidly and cover over the denuded area. The change may progress until the layer of mesothelium is several cells thick and even in this state it is ample to prevent the development of adhesions. In long continued irritations connective-tissue proliferation takes place and small outgrowths appear on the parenchymatous organs, but even here the changed mesothelium rapidly covers the advancing core of blood vessels and connective-tissue stroma, preventing the development of adhesions.

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EXPLANATION OF FIGURES

All the figures are from micro-photographs of sections taken perpendicularly to the surfaces of the organs. The negatives have not been retouched.

Fig. 1.—Spleen of rabbit which has been irritated for 6 days by intraperitoneal injections of a carmine suspension. ×250.

Fig. 2.—Ovary of rabbit which has been vitally stained with trypan blue by intravenous injections. The germinal epithelium shows the blue as small dark droplets collected about the inferior poles of the nuclei. ×250.

Fig. 3.—Liver of cat which has received repeated intraperitoneal injections of trypan blue, 1% in physiological saline, during a period of 10 days. Note that the cells are more rounded than in Fig. 1. ×230.

Fig. 4.—Spleen of cat which has received repeated injections of laked blood intraperitoneally for two weeks and during the same period has received intravenous injections of trypan blue. Note the clear vacuoles in the bases of the cells; these contain small granules of trypan blue. ×230.

Fig. 5.—Spleen of cat which has received laked rabbit's blood every other day over a period of 26 days. This shows the maximal stage of proliferation obtained without the formation of any adhesions. ×250.

Fig. 6.—Spleen of cat which has received laked blood every other day for a period of 20 days. Here the proliferation is not quite so extensive as shown in Fig. 5. ×230.

Fig. 7.—Liver of cat that has received laked blood over a period of 25 days. This shows beginning proliferation of the subjacent blood-vessels and connective-tissue stroma. ×230.

Fig. 8.—Liver of cat that has received laked blood over a period of 26 days (same animal as in Fig. 5), showing further stage of change similar to that shown in Fig. 7. ×230.

Fig. 9.—Diaphragm of rabbit which has received 3 doses of laked blood on successive days. This shows the low cuboidal mesothelium which is more typical of early changes on body-wall than diaphragm. Section taken near point of attachment of the muscular part of the diaphragm to the body wall. ×1130.

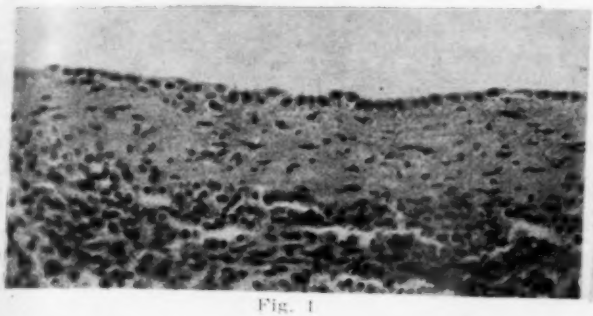

Fig. 1

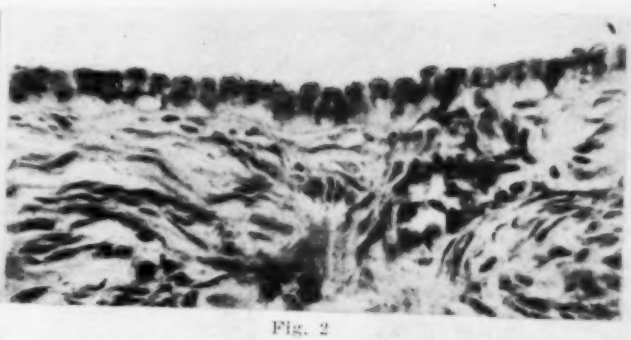

Fig. 2

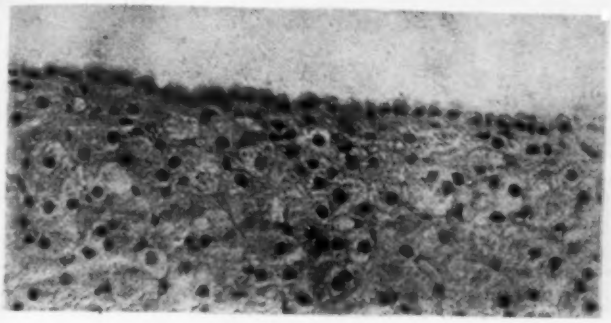

Fig. 3

Fig. 4

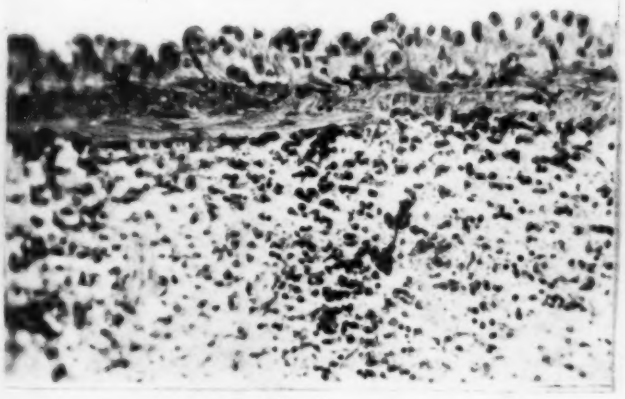

Fig. 5

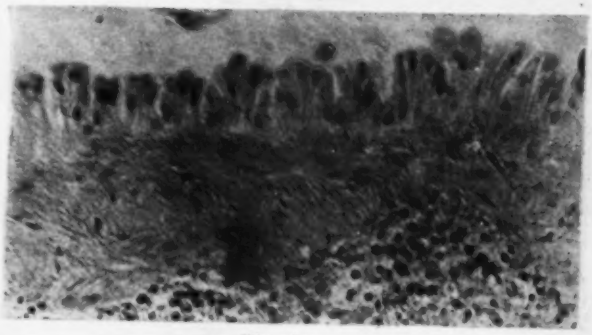

Fig. 6

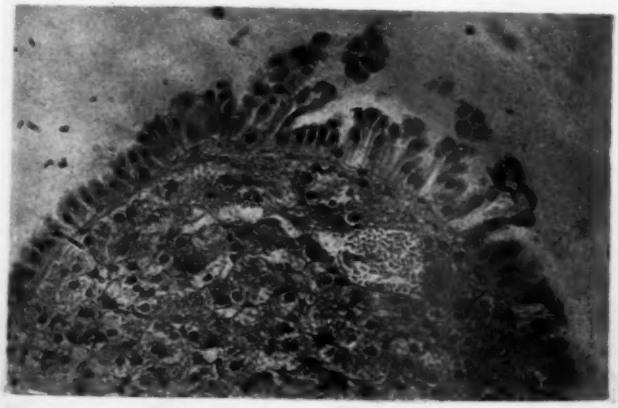

Fig. 7

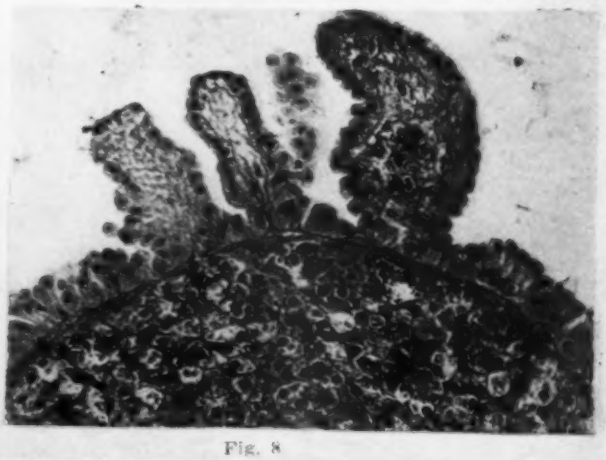

Fig. 8

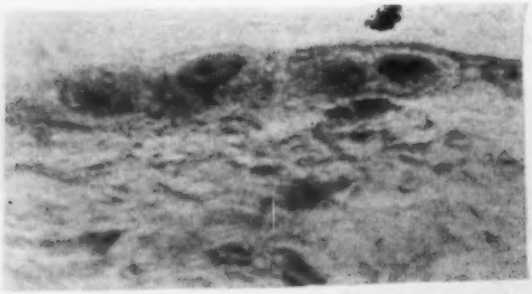

Fig. 9

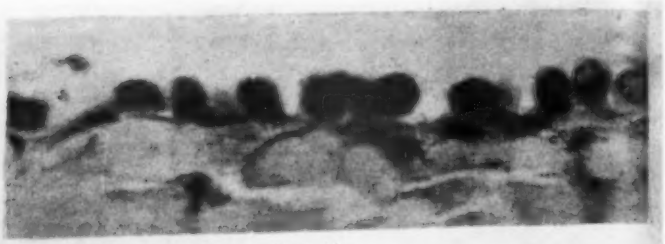

Fig. 10

Fig. 11

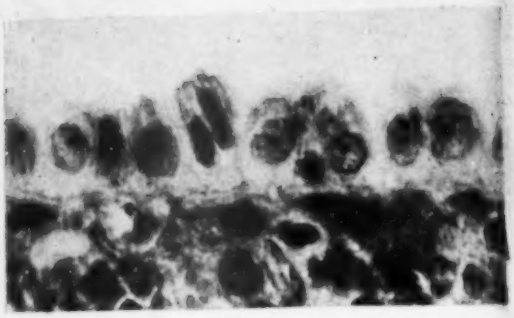

Fig. 12

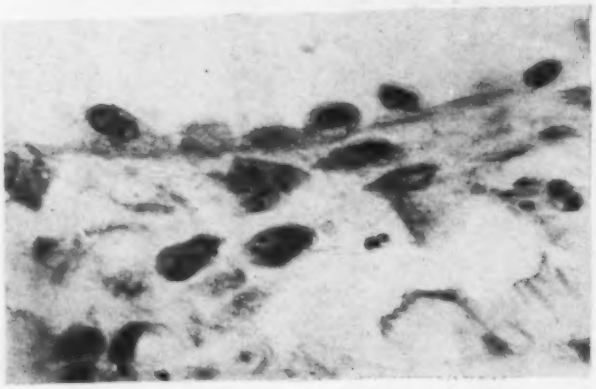

Fig. 13

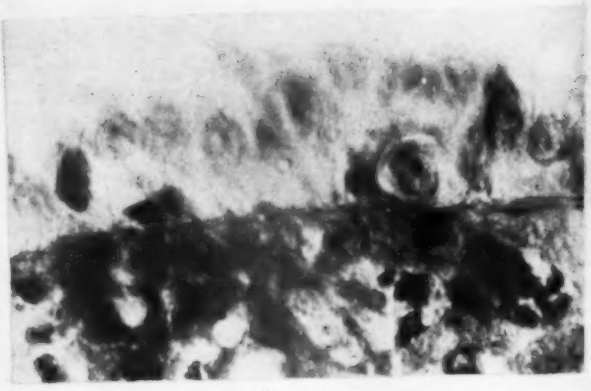

Fig. 14

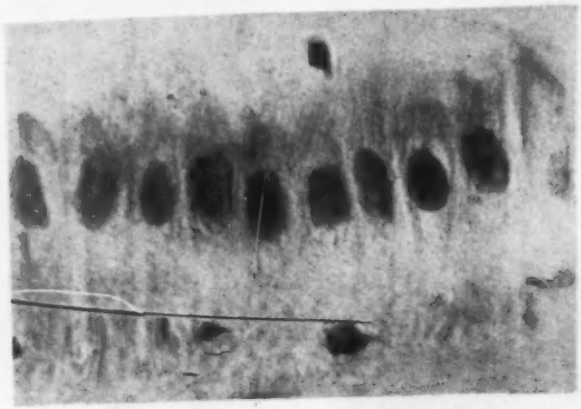

Fig. 15

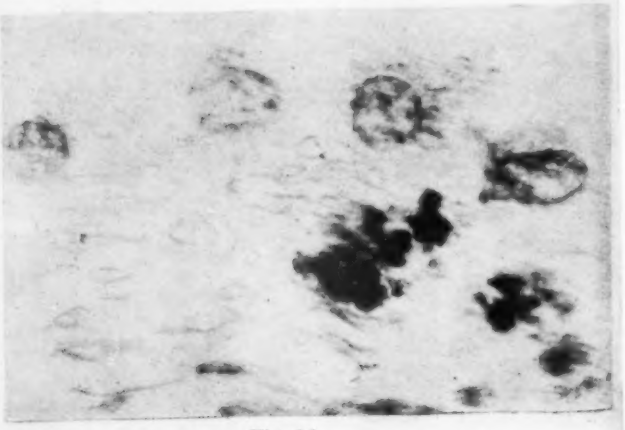

Fig. 16

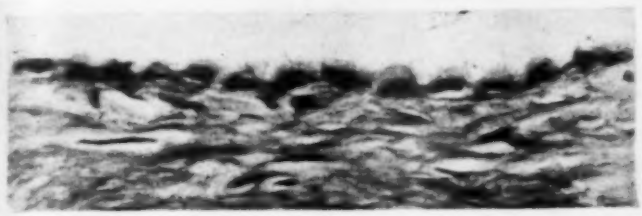

Fig. 17

Fig. 18

Fig. 19

Fig. 20

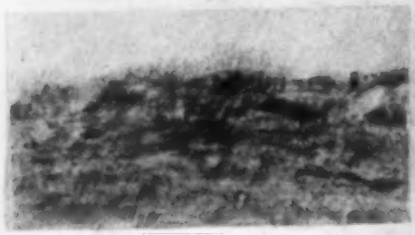

Fig. 21

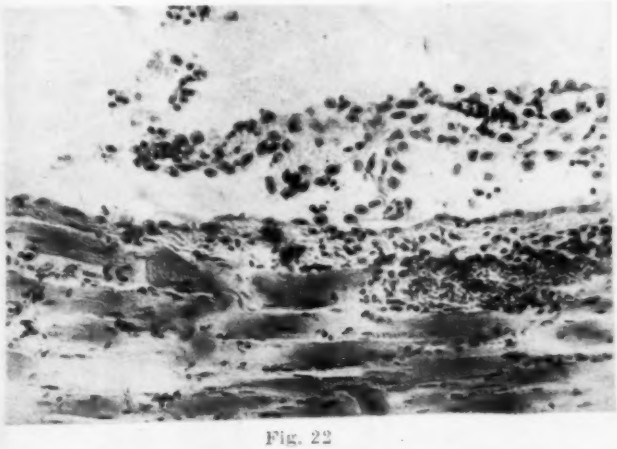

Fig. 22

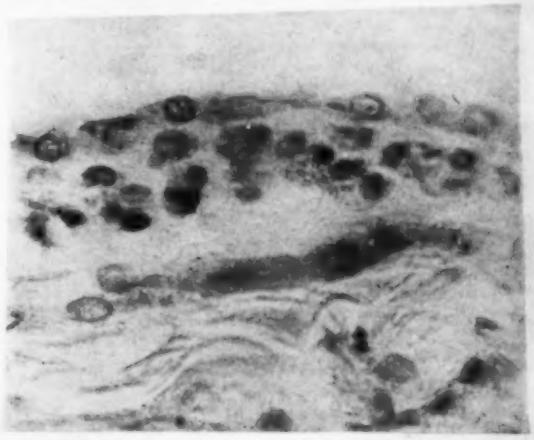

Fig. 23

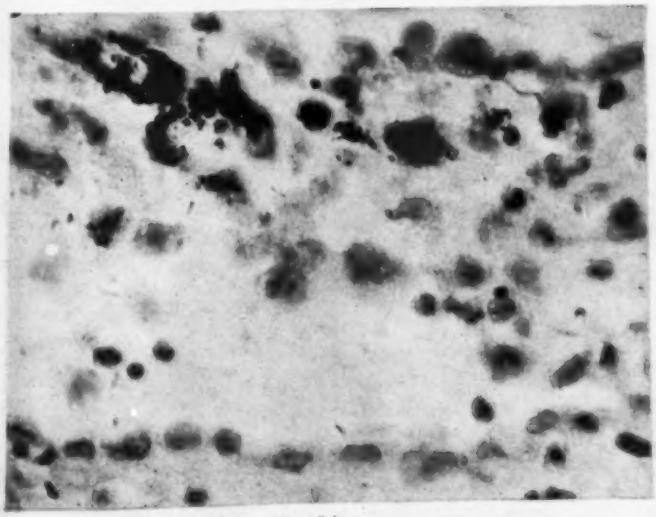

Fig. 24

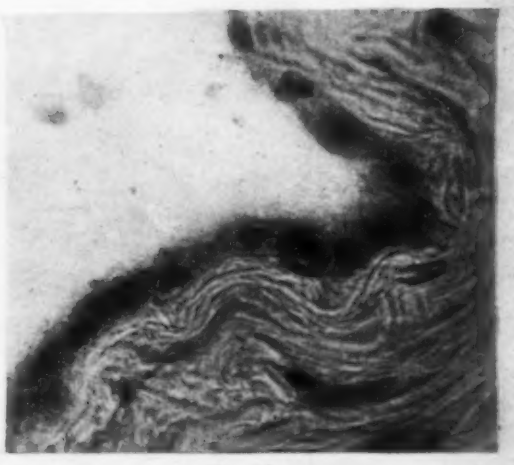

Fig. 25

Fig. 10.—Section from tendinous part of the diaphragm of a cat that has received 6 injections of laked blood intraperitoneally. Note the contrast between these rounded cells and those of Figs. 9 and 25. ×775.

Fig. 11.—Liver of cat that has received 2% Niagara blue solution intraperitoneally for a period of 3 weeks and laked blood during the last 3 days. Note the basal cells. ×1180.

Fig. 12.—Liver of cat that has received laked blood for 18 days intraperitoneally. Note the binucleate cell with nuclei parallel to the long axis of the cell. ×1180.

Fig. 13.—Omentum of cat that has received laked blood intraperitoneally for 24 days. Note cellular division to left of center. ×680.

Fig. 14.—Liver of same animal as shown in Fig. 11. ×1180.

Fig. 15.—Spleen of cat from same animal as figured in 11 and 14. ×1300.

Fig. 16.—Omentum of rabbit that has received laked blood intraperitoneally and trypan blue intravenously for 12 days. Note the two clasmatocytes loaded with dye just beneath the serosal cells. ×1500.

Fig. 17.—Section from rabbit's spleen taken from region adjacent to an infarct. ×680.

Fig. 18.—Mesentery of cat showing vacuolization of a mesothelial cell about the center of the picture. This often occurs in advanced stages of irritation. ×280.

Figs. 19, 20, 21.—Spleen of rabbit vitally stained by intravenous injections of carmine. The carmine is collected in small masses of granules adjacent to the nuclei. ×1200.

Fig. 22.—Diaphragm of rabbit that has received whole blood intraperitoneally for 15 days. Shows the covering of exudate by extension of the mesothelial layer. ×280.

Fig. 23.—From same section as Fig. 22, showing two layers of mesothelium, one covered by the exudate and the other external to it. ×775.

Fig. 24.—Higher magnification of the extreme right of Fig. 22. ×600.

Fig. 25.—Section from body-wall of same cat from which Fig. 5 was taken. ×720.

ON THE MODE OF TRANSMISSION OF THE STREPTOCOCCI ASSOCIATED WITH TONSILLITIS

By A. L. BLOOMFIELD and A. R. FELTY

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During the study of an outbreak of acute streptococcic tonsillitis epidemiological evidence was forthcoming which indicated that extremely close association was necessary before susceptible people acquired the disease from carriers or from cases.[1] In the group under consideration, namely, the pupil nurses of the Johns Hopkins Hospital Training School, it appeared that infection took place largely or entirely during hours of recreation when the students were intimately gathered in considerable numbers in the small dormitory rooms. It seemed desirable, therefore, to determine more in detail the mode of spread of the virus and to obtain quantitative data on the importance of various types of "exposure," such as close association without actual contact, and contact, direct and indirect, of different degree.

In approaching this problem it was necessary to consider the various recognized modes of transmission of bacterial infection by air (droplet), by contact, and by fomites. This matter has been threshed out in a general way so often and to such good effect that we might question the fruitfulness of further study in relation to any particular disease, but certain special conditions obtain in tonsillar infection which make it necessary, as will be pointed out, to depart from general principles, and the results of the inquiry actually show that the mode of transmission of tonsillitis is a highly restricted one.

Studies on the dissemination of bacteria in the upper air passages made several years ago indicated that the mouth could not be regarded as a culture tube in which foreign bacteria grew diffusely and uniformly, but that they were for the most part confined to certain circumscribed localities. This was especially true of the tonsil. In the case of a "tonsil carrier," for example, of hemolytic streptococcus, staphylococcus aureus or Friedländer's bacillus, the bacteria in question could be obtained regularly in culture from the site of the focal infection but to no great extent from adjacent regions and practically never from the anterior portion of the mouth. Illustrative protocols may be found in a previous paper.[2] A further investigation brought out the fact that there was in the mouth a highly organized eliminative mechanism by means of which bacteria were rapidly swept back to the pharynx,[3] and in this observation seemed to lie the explanation of the absence of generalized dissemination throughout the oral cavity from a localized tonsillar focus.

The previous studies had all been made with chronic carriers, but it seemed probable that similar conditions would obtain with acute tonsillitis and that despite the vast number of streptococci in the posterior part of the buccal cavity and pharynx there might be considerable opposition to their forward progress and ejection to the outside world. The implication as regards transmission is obvious.

To obtain preliminary information simultaneous differential cultures were made from a case of tonsillitis during the acute stage.

Expt. I.—Patient C.

Site of Culture	Hemolytic Strep.	Other Bacteria
Pharynx and tonsils	∞ *	∞ A few
Lips	0	∞ Mouth Flora
Tongue	0	∞ Mouth Flora
Buccal Mucosa (right)	0	∞ Mouth Flora
Buccal Mucosa (left)	0	∞ Mouth Flora

* ∞ —innumerable.

This observation, then, was in accord with those previously made on carriers, and showed that even during an acute infection when the tonsils and pharynx were swarming with hemolytic streptococci none could be obtained from the anterior part of the mouth. With this as a basis more systematic studies were made which are herewith presented.

Aerial (Droplet) Transmission in Tonsillitis

Aerial transmission of bacteria in the restricted sense, that is to say, the wafting of bacteria for considerable distances without any vehicle of conveyance, is now a matter of historical interest. The dissemination of organisms by droplets of saliva or mucus ejected especially by coughing or sneezing still holds a definite place in epidemiology, although perhaps not as important a one as at first seemed probable. The subject is too familiar to all to require review, but one may refer to the classical and ingenious experiments of Flügge and his associates which have been assembled in monographic form,[4] and to other references which can be found in Chapin's book on infection.[5] The usual type of experiment has consisted of implanting some distinctive organism such as B. prodigiosus in the mouth of the experimenter and exposing plates at various distances in order to determine how far the bacteria are ejected by conversation, coughing, sneezing or other manoeuvers. In one connection or another this experiment has been repeated often enough to show that while few or no bacteria leave the mouth during quiet conversation vigorous coughing may disseminate them to a distance of at least 9 meters depending upon humidity, air currents, etc. The quantitative relations can be best brought out by reproducing a protocol from a recent paper by Doust and Lyon [6] (Table I).

But while the potentiality of droplet transmission is generally recognized, bitter controversies have existed in the past as to the actual practical importance of this phenomenon in the dissemination of bacteria. At the present time opinions are still divided, but most students of infection feel that spraying is a real factor in the spread of respiratory disease.

In considering the principle of droplet infection in relation to the spread of tonsillitis we were unable to find in the literature any experiments on the expulsion of bacteria from a local area of infection in the tonsil region. When prodigiosus was employed, the experimenter's mouth was rinsed with the culture so that no differential data could be obtained, and the same applied to coughing tuberculous patients. A series of experiments was therefore done to determine whether bacteria implanted on local areas in the posterior buccal cavity were ejected by coughing and other procedures with the same facility as obtains when the lip and tongue are the site of the organisms.

TABLE I.

Projection of B. prodigiosus from the mouth on agar plates during conversational speech, loud speech, and coughing.*

	Distance of Plates from Operator in Feet									
	1	2	3	4	5	6	7	8	9	10
Speaking in ordinary conversational tone for 5 minutes	1	0	0	0	0	0	–	–	–	–
	0	0	0	0	0	0	–	–	–	–
	0	1	0	0	0	0	–	–	–	–
	13	2	0	0	0	0	–	–	–	–
Speaking in ordinary conversational tone for 30 minutes	16	0	0	0	0	0	–	–	–	–
Speaking in loud tone for 5 minutes	72	0	0	0	0	0	–	–	–	–
	49	3	2	0	0	0	–	–	–	–
	0	0	0	0	0	0	–	–	–	–
	115	5	5	1	0	0	–	–	–	–
Coughing for 5 minutes	387	381	245	76	26	4	–	–	–	–
	–	–	–	248	116	94	32	18	17	8
	24[illegible]	64	19	[illegible]	3	1	–	–	–	–
	–	–	4	6	3	1	2	0	0	0

* Number of colonies of B. prodigiosus on each plate in figures; dash indicates plates not exposed.

Methods

Twenty-four-hour agar slants of colon bacillus were used. Solid masses of growth (usually about half a slant) were smeared on the selected site. During the ensuing procedures plates of 25 per cent bile agar were held at various distances in front of the mouth. At the end of the experiment a second layer of the medium was poured on the plates which were then gently agitated so as to spread the colonies and allow better quantitative estimations. The bile agar furnished a satisfactory selective medium, inasmuch as practically all the mouth bacteria were inhibited, whereas the implanted colon bacilli throve. After incubation for 24 hours the results were recorded.

Expt. II.—October 19, 1923. Healthy subject. Mouth and throat clinically negative. Tonsils small and clean. The entire growth from a 24-hour agar slant of B. coli was swabbed over the posterior part of the tongue, palate and tonsils.

Plate No.	Procedure	Plate Exposed		No. Colonies B. coli Obtained
		Distance	Time	
1	Conversation	1 foot	1 minute	0

Expt. II.—Continued.

2	Conversation	6 feet	1 minute	0
3	Vigorous cough	1 foot	1 minute	0*
4	Vigorous cough	6 feet	1 minute	0
5	Sneezing	1 foot	15 seconds	0*
6	Sneezing	6 feet	15 seconds	0
7	Control culture from site of inoculation.			∞ B. coli
	The lips, gums and anterior tongue were then swabbed with B. coli			
8	Conversation	1 foot	1 minute	0
9	Conversation	6 feet	1 minute	0
10	Vigorous cough	1 foot	1 minute	About 100*
11	Vigorous cough	6 feet	1 minute	0
12	Sneezing.....	1 foot	15 seconds	∞ B. coli*
13	Sneezing	6 feet	15 seconds	0
14	Control culture	6 feet	15 seconds	∞ B. coli

* Macroscopic spraying of these plates.

Comment.—Bacteria implanted on the tonsils and posterior tongue were not expelled even by violent coughing and sneezing. After implantation on the lips and anterior tongue they were readily obtained.

Expt. III.—October 22, 1923. Healthy subject. Mouth and throat clinically negative. Same methods as in Expt. I. Soft palate, posterior pharynx and tonsils swabbed with solid masses of 24-hour growth of B. coli.

Plate No.	Procedure	Plate Exposed		No. of Colonies of B. coli Obtained
		Distance	Time	
1	Conversation	1 foot	1 minute	0
2	Conversation	6 feet	1 minute	0
3	Cough	1 foot	1 minute	0*
4	Cough	6 feet	1 minute	0*
5	Sneezing	1 foot	15 seconds	0
6	Sneezing	6 feet	15 seconds	0
7	Control culture posterior pharynx.			∞ B. coli
	Anterior tongue and lips were then swabbed with B. coli			
8	Conversation	1 foot	1 minute	0
9	Conversation	6 feet	1 minute	0
10	Cough	1 foot	1 minute	∞ B. coli*
11	Cough	6 feet	1 minute	0
12	Sneezing.....	1 foot	1 minute	∞ B. coli*
13	Sneezing	6 feet	1 minute	0
14	Control culture anterior tongue			∞ B. coli

* Macroscopic spraying.

Comment.—Results similar to those in Expt. I.

Expt. IV.—October 23, 1923. Healthy subject. Mouth and throat clinically normal. Same methods as in Expt. I. Mass of solid 24-hour growth of B. coli implanted on uvula.

Plate No.	Procedure	Plate Exposed		No. of Colonies of B. coli Obtained
		Distance	Time	
1	Conversation	6 inches	1 minute	0
2	Coughing	6 inches	1 minute	0
3	Sneezing	6 inches	15 seconds	0
4	Control culture from uvula			∞ B. coli

Discussion of Experiments II to IV.—The main point is that procedures which eject bacteria implanted on the lips and anterior part of the mouth fail to do so when the organisms are swabbed on the pharynx, soft palate, tonsils or uvula. It is impossible, therefore, to transfer conclusions from the recorded experiments on droplet expulsion, which were done after the whole mouth had been rinsed with prodigiosus culture, to the problem of tonsillitis—a disease in which the virus is localized in the tonsillar region. The next experiments, therefore, were done with clinical cases of tonsillitis and scarlet fever.

METHODS

The same general methods were used as in the B. coli experiments. Thin plates of 5% blood agar were poured and, after exposure, another layer of blood agar was added. Colonies of Beta hemolytic streptococcus were readily identified and counted.

Expt. V.—Patient R. Acute follicular tonsillitis Control culture from tonsils yielded innumerable Beta hemolytic streptococci in practically pure culture. The patient was in a hospital room for two days.

Plate No.	Procedure	Exposure of Plate		No. of Colonies Recovered	
		Distance	Time	Hemol. Strep.	Other Bacteria
1	Conversation	1 foot	1 minute	0	9 cols. (air)
2	Laughing	1 foot	1 minute	0	0
3	Cough (1)	1 foot		0	1 col. (air)
4	Coughing	1 foot	30 seconds	0	0
5	Coughing	6 feet	30 seconds	0	2 cols. (air)
6	Inspection of patient's throat	Plate held 30 sec. in front of examiner's mouth		0	0
7	Sneezing	1 foot	15 seconds	0	About 20 colonies mostly Strept. salivarius
8	Sneezing	6 feet	15 seconds	0	0

Comment.—The culture from tonsils showed innumerable Beta hemolytic streptococcus but none were recovered by the above procedures although coughing and sneezing led to the ejection of normal mouth bacteria, presumably from the anterior part of the buccal cavity. The results are in accord with the colon bacillus experiments.

Expt. VI.—P. C. Scarlet Fever, third day. Control culture from tonsils and pharynx yielded innumerable Beta hemolytic streptococci in pure culture.

Plate No.	Procedure	Exposure of Plate		No. of Colonies Recovered	
		Distance	Time	Hemol. Strep.	Other Bacteria
1	Conversation	1 foot	1 minute*	0	0
2	Vigorous coughing	3 inches	1 minute*	4	∞ cols. normal mouth flora
3	Sneezing	3 inches	1 minute*	30	∞ cols. normal mouth flora

* Macroscopic spray on plates.

Comment.—Whereas innumerable bacteria of the normal mouth flora were ejected, only a very few hemolytic streptococci were obtained by these violent procedures.

Expt. VII.—Patient G. Acute tonsillitis, third day. Control culture from tonsils—innumerable Beta hemolytic streptococci in practically pure culture.

Plate No.	Procedure	Plate Exposed		No. of Colonies Recovered	
		Distance	Time	Hemol. Strep.	Other Bacteria
1	Conversation	6 inches	1 minute	0	1 col. (air)
2	Coughing*	6 inches	1 minute	0	10 cols. mouth flora
3	Sneezing*	6 inches	15 seconds	0	∞ cols. mouth flora

* Macroscopic spraying of plate.

Comment.—No hemolytic streptococci obtained by these procedures.

Expt. VIII.—E. A. Acute tonsillitis. Control culture from tonsils—innumerable Beta hemolytic streptococci in practically pure culture.

Plate No.	Procedure	Exposure of plate		No. of Coloines Recovered	
		Distance	Time	Hemol. Strep.	Other Bacteria
1	Conversation	6 inches	1 minute	0	3 cols. (air)
2	Cough	6 inches	1 minute	0	20 cols. (misc.)
3	Sneezing	6 inches	15 seconds	0	∞ mouth flora

Comment.—No hemolytic streptococci obtained by these procedures.

Discussion of Experiments V to VIII.—It is seen that these experiments are in accord with the colon implantations. In these infections—tonsillitis and scarlet fever—in which the bacteria are confined to the tonsillar region even the most violent coughing and sneezing fail to eject more than an occasional hemolytic streptococcus. It must be recalled that tonsillitis is not a coughing disease and without artificial manoeuvers it seems unlikely that the causal bacteria are ejected in droplets.

The question arose, however, whether streptococci are not from time to time brought forward into the anterior part of the mouth by hawking and expectoration and whether then they might not be expelled by the droplet route. The following experiments were devised to test this possibility.

Expt. IX.—Normal subject. The tonsils and pharynx were swabbed with solid growth of B. coli. Plates exposed during the usual procedures of conversation, coughing and sneezing, were negative. The patient was then instructed to hawk and to expectorate, and thereafter to cough and to sneeze.

Plate No.	Procedure	Plate Exposed		No. of Colonies of B. coli Obtained
		Distance	Time	
1	Culture of hawkings			Several hundred colonies B. coli
2	Violent coughing	6 inches	1 minute	0
3	Violent sneezing	6 inches	1 minute	0
4	Control culture site of inoculation			∞ B. coli
5	Control culture anterior tongue and lips			0

Comment.—Although many colon bacilli were ejected in hawkings there apparently was no marked contamination of the anterior mouth and the manoeuver was not followed by droplet ejection of colon bacilli.

Expt. X.—Normal subject. The tonsils and pharynx were swabbed with solid growth of B. coli. Plates exposed during the usual procedures of coughing, speaking, and sneezing were negative. The subject then gargled with water and expectorated.

Plate No.	Procedure	Plate Exposed		No. of Colonies of B. coli Recovered
		Distance	Time	
1	Culture of expectorated material			Several hundred colonies B. coli
2	Violent coughing	6 inches	1 minute	0
3	Violent sneezing	6 inches	15 seconds	0
4	Control culture from site of inoculation			∞ mouth flora
5	Control culture from anterior tongue and lip			0

Comment.—Gargling and expectoration yielded many colonies of B. coli, but contamination of the anterior mouth in this way did not lead to droplet ejection of the bacteria.

Similar experiments were next done with cases of tonsillitis and scarlet fever.

Expt. XI.—P. C., scarlet fever. Throat culture—innumerable Beta hemolytic streptococci. Violent coughing and sneezing yielded, respectively, only 4 and 30 colonies of hemolytic streptococci, although there were innumerable colonies of normal mouth flora. The patient then hawked, churned the hawkings around the anterior mouth for two minutes, and expectorated.

Plate No.	Procedure	Plate Exposed		Number of Colonies Obtained	
		Distance	Time	Hemol. Strep.	Other Bacteria
1	Culture of hawkings			Many B. H. S. about 1% plate	∞ mouth flora
2	Violent cough	6 inches	1 minute	5	∞ mouth flora
3	Violent sneezing	6 inches	15 seconds	30	∞ mouth flora

Comment.—Results similar to those in experiments VIII and IX with B. coli.

Expt. XII.—G. Acute follicular tonsillitis. Throat culture—innumerable Beta hemolytic streptococci. Violent coughing and sneezing yielded no hemolytic streptococci, although many colonies of mouth flora were obtained. The patient then hawked and expectorated.

Plate No.	Procedure	Plate Exposed		No. of Colonies Obtained	
		Time	Distance	Hemol. Strep.	Other Bacteria
1	Culture of hawkings			1% of plate Few B. H. S.	∞ mouth flora
2	Conversation	1 minute	6 inches	0	0
3	Coughing*	1 minute	6 inches	0	Many colonies mouth flora
4	Sneezing*	15 seconds	6 inches	0	∞ mouth flora

* Macroscopic spraying.

Comment.—Results similar to those in preceding experiments.

Expt. XIII.—E. Acute follicular tonsillitis. Throat culture—innumerable Beta hemolytic streptococci. Violent coughing and sneezing yielded no hemolytic streptococci although many colonies of mouth flora were obtained. The patient then hawked and expectorated.

Plate No.	Procedure	Plate Exposed		No. of Colonies Obtained	
		Time	Distance	Hemol. Strep.	Other Bacteria
1	Hawkings			Many (5% of plate)	∞ mouth flora
2	Conversation	1 minute	6 inches	0	0
3	Coughing	1 minute	6 inches	3	100 cols. mouth flora
4	Sneezing	15 seconds	6 inches	5	∞ cols. mouth flora

Comment.—A very few hemolytic streptococci expelled by violent procedures.

Discussion of Experiments IX to XIII.—It was shown that when the bacteria were primarily localized in the tonsillar and pharyngeal regions such procedures as hawking and gargling led to ejection of the organisms in expectorated material. Contamination of the anterior mouth in this way was followed by droplet expulsion of only an occasional hemolytic streptococcus. Probably the consistence of the material was responsible.

In *summary* then it was not possible even by the most forced manoeuvers to demonstrate that streptococci in tonsillitis (and scarlet fever) are ejected to any degree by droplets. The conclusion seems justified that aerial (droplet) infection plays no part in the spread of this disease.

Several other experiments were done by way of control to see whether hemolytic streptococci could be recovered from the air in the vicinity of patients.

Expt. XIV.—Patient C. Acute follicular tonsillitis—convalescent. Control culture from tonsils yields innumerable Beta hemolytic streptococci, practically pure. Six large blood agar plates were suspended vertically one foot in front of the patient's mouth for two hours. During this time she talked, laughed and acted as one would during ordinary conversation.

Result.—No hemolytic streptococci were obtained, but there were about 200 colonies mostly of air bacteria per plate.

Expt. XV.—Patient P. C. Scarlet fever. Control culture from tonsils and pharynx—innumerable Beta hemolytic streptococci. Seven blood agar plates were exposed for two hours within three feet of the patient's mouth—on the bed, bedside-table, etc. During the experiment he conversed and coughed violently and frequently.

Result.—No hemolytic streptococci.

Expt. XVI.—Patient G. Acute tonsillitis. Same procedure as in Expt. XIV.

Result.—About 100 colonies per plate—mostly air bacteria. On one plate there is a cluster of about 40 colonies of Beta hemolytic streptococci.

Expt. XVII.—Patient E. Acute tonsillitis. Same procedure as in Expt. XVI.

Result.—About 100 colonies per plate—air bacteria and normal mouth flora. Only one colony of Beta hemolytic streptococci.

These experiments indicate that only occasionally can streptococci be recovered from the air in the vicinity of the patient. They seem to bring further evidence against aerial infection.

Transmission of Tonsillitis by Contact

With droplet infection ruled out it seemed probable that contact must be the means by which the disease is spread. Such contact might be direct as, for example, by kissing, or indirect by means of objects freshly contaminated such as spoons, forks, toilet articles, etc. The next group of experiments was designed to test this possibility. Experiments were first done with colon bacillus implantations and later with cases of tonsillitis and scarlet fever.

Several procedures were used. To imitate direct contact the subjects were instructed to kiss open Petri dishes. Objects such as wooden spatulae, spoons, etc., were then placed in mouth and later cultured.

Expt. XVIII.—Normal subject. The palate, posterior pharynx and tonsils were swabbed with B. coli.

Plate No.	Procedure	No. Colonies B. coli Obtained
1	Kissed plate	0
2	Culture from spatula placed in mouth for several minutes.	0
	The patient then hawked and expectorated.	
3	Culture of hawkings	∞ B. coli
4	Kissed plate	∞ B. coli
5	Culture from spatula placed in mouth	∞ B. coli

Comment.—Under the same conditions which failed to lead to droplet infection, bacteria were expelled by kissing and on foreign objects placed in the mouth.

Expt. XIX.—Normal subject. The tonsils were swabbed with B. coli.

Plate No.	Procedure	No. Colonies B. coli Obtained
1	Kissed plate	0
2	Spatula culture	25 colonies
	The subject then hawked and expectorated	
3	Culture of hawkings	Several hundred cols. B. coli
4	Kissed plate	0
5	Spatula culture	2

Comment.—Results similar to those in Expt. XIV, but less striking.

Expt. XX.—Normal subject. Tonsils swabbed with B. coli.

Plate No.	Procedure	No. Colonies B. coli Obtained
1	Kissed plate	0
2	Spatula culture.	0
	Patient then gargled with water and expectorated.	
3	Culture expectorations	Many B. coli
4	Kissed plate	0
5	Spatula culture	0

Expt. XXI.—Patient G. Acute tonsillitis. Culture from tonsils—innumerable Beta hemolytic streptococci, practically pure.

Plate No.	Procedure	No. of Colonies Obtained	
		Hemol. Strep.	Other Bacteria
1	Kissed plate	0	Several thousand colonies, mostly Staph. albus
2	Spatula culture.	0	∞ colonies (mouth flora)
	Patient then hawked and expectorated		
3	Culture expectorations	Many	∞ colonies (mouth flora)
4	Kissed plate	0	∞ colonies Staph. albus and mouth flora
5	Spatula culture	0	∞ colonies mouth flora

Expt. XXII.—Patient E. Acute tonsillitis. Culture from tonsils—innumerable Beta hemolytic streptococci—practically pure.

Plate No.	Procedure	No. of Colonies Obtained	
		Hemol. Strep.	Other Bacteria
1	Kissed plate	0	About 100 colonies—Staph. albus and Strep. viridans
2	Spatula culture.	0	∞ colonies mouth flora
	Patient then hawked and expectorated		
3	Culture expectorations	Many	∞ colonies mouth flora
4	Kissed plate	1	About 50 colonies, mostly Staph. albus
5	Spatula culture	Many (but less than 1% plate)	∞ colonies mouth flora

Discussion of Experiments XVIII to XXII.—Bacteria from the tonsillar region are not ejected with droplets, but they may be brought forward into the mouth by hawking and be ejected by expectoration. Foreign objects placed in the mouth may also be contaminated.

It seems evident then that direct contact must be the method by which tonsillitis is transmitted and this is in harmony with the clinical observation that close association is essential to the spread of the disease. To what extent fomites or dust transmission also play a part is a question which deserves consideration. The possibility is a real one and its practical importance must depend on how long hemolytic streptococci survive in the outside world. Ample data of general import are available and all would indicate that save under unusual conditions such as preservation of dried secretions in dark places the organisms die promptly.[5] Even if viable, they must be in a latent stage of vegetative activity and probably could not produce disease except under the rarest of circumstances.

General Discussion

The experiments which have been outlined indicate that aerial (droplet) infection plays no part in the spread of streptococcic tonsillitis and that dust and fomites are also of little consequence. The disease it would seem, is transmitted by contact, which is either direct from person to person or through the mediation of fresh secretions or freshly contaminated objects. Even so there is already a fairly effective barrier to the ejection of streptococci from the tonsillar region as indicated by the need of forced procedures such as hawking to bring them forward into the mouth.

In the light of these facts it is of interest to reconsider some of the phenomena of the observed spread of tonsillitis. In the group which we studied during the winter of 1922-1923 there were 34 people who were regarded as highly susceptible. Although they were in intimate and constant association with at least an equal number of carriers, only 21 (or 62%) developed tonsillitis and, furthermore, the cases appeared singly over a period of six months.[7] On the other hand, the readiness with which tonsillitis follows if streptococci actually reach the upper air passages is evident from recorded instances of experimental infection which have followed inoculations of nasopharyngeal washings into volunteers in attempts to transmit influenza. These experiments which were carried on under the auspices of the U. S. Public Health Service [8] during the pandemic of 1918-19, are discussed by Ritchie [9] in another connection, but have a very direct bearing on the present problem. Fresh nasal and pharyngeal secretions were collected from cases of influenza and instilled into the noses and mouths of healthy young men. Preliminary cultures of the material showed that in a good many cases hemolytic streptococci of the Beta type were present and in actual fact a considerable number of the volunteers developed tonsillitis within 36 to 72 hours.

TABLE II.

Transmission of tonsillitis by inoculation of Throat secretions. (Compiled from Hygienic Laboratory Bulletin No. 123.)

Exp. No.	Material	Number of Volunteers	Cases of Tonsilitis Following Inoculation
Boston series			
1	Neg. for H. Strep.	10	0
3	Neg. for H. Strep.	10	1
8	Neg. for H. Strep.	9	0
		Total 29	1
2	Pos. for H. Strep.	10	1
4	Pos. for H. Strep.	10	0
5	Pos. for H. Strep.	4	2
6	Pos. for H. Strep.	4	0
7	Pos. for H. Strep.	10	5
9	Pos. for H. Strep.	15	3
		Total 53	11
San Francisco series			
1	Neg. for H. Strep.	10	0
3	Neg. for H. Strep.	10	0
		Total 20	0
2	Pos. for H. Strep.	4	1
4	Pos. for H. Strep.	4	2
5	Pos. for H. Strep.	2	0
		Total 10	3

Summary

Total volunteers inoculated with negative material..... 49
Cases of tonsillitis..... 1
Total volunteers inoculated with positive material..... 63
Cases of tonsillitis..... 14

An analysis of the reported experiments is of great interest (Table II) and brings out many important facts. Among 64 men receiving material "positive" for hemolytic streptococcus 14 (22%) developed tonsillitis. In a group of our own, 39 of 102 people (38%) were regarded as susceptible. If the same figure can be applied to the naval experimental group one might suppose that there were among them 24 susceptibles. Of these, 14 (60%) developed tonsillitis, a figure which corresponds strik-

ingly with the incidence in our group during an entire season. Furthermore, in Experiments II and IV, three of eight men who had been inoculated, or nearly 40%, came down, whereas twelve uninoculated controls who were isolated with them all remained well. Again, in the series of experiments in which material "negative" for hemolytic streptococcus was used, one case of tonsillitis developed among 49 men, although there was close contact in the group and a high percentage of carriers.

Finally, it is notable that milk-borne outbreaks of tonsillitis involve a high percentage of those who drink the infected milk, although there is no evidence of secondary spread from case to case.

All the evidence, therefore, indicates the ease with which susceptibles may be infected, provided the streptococci enter the air passages in a vegetative state and in large numbers, and any high degree of apparent resistance must be ascribed to the difficulties which stand in the way of natural transmission from person to person.

Approaching the matter from a different angle Dudley,[10] in a study of an outbreak of tonsillitis and scarlet fever in a boys' boarding school, was forced to the conclusion that spread must be due to contact infection and suggested promiscuous chewing of penholders by the boys as the means of transfer.

Conclusions

The question of mode of transmission of tonsillitis may then be summarized as follows: Aerial (droplet) infection plays no part, because the causal organisms are located in a region from which they are not eliminated by this method. Furthermore, since only few and occasional hemolytic streptococci come forward into the mouth at all, contamination of objects even if placed in the mouth is relatively infrequent. Expectorated hawkings seem the most probable vehicle by means of which the bacteria are discharged.

Hence transmission must be by direct contact with the patient or with freshly contaminated objects.

The bearings of these findings on the practical problem of isolation are obvious. Furthermore, if scarlet fever is finally shown to be caused by hemolytic streptococci—as seems quite likely—the same principles will hold in that disease.

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ON THE PECULIAR MORPHOLOGY OF A MALARIAL PARASITE IN A CASE OF CHRONIC MALARIA IN THE TEMPERATE ZONE

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Introduction

Since the appearance, in 1914, of a paper by Stephens[1] entitled "A New Malaria Parasite of Man," there has been much discussion concerning the exact nature of this parasite and quite a good deal of adverse criticism of Stephens' conclusions. He called this parasite Plasmodium tenue because it was very amoeboid, because the cytoplasm was scanty and because the nuclear chromatin was proportionately great for the volume of the parasite. Stephens was uncertain whether the parasite enlarged the infected cell. He described three pigmented leucocytes but observed Schüffner's granules in only one infected cell.

Balfour and Wenyon[2] severely criticized Stephens' work. They stated correctly that "Stephens based his conclusions on the morphological peculiarities of a malarial parasite which he found in a single blood film." These authors pointed out that Plate VII of the Third Report of the Wellcome Tropical Research Laboratories (1908) pictured a malarial parasite closely resembling, if not identical with, Stephens' parasite. In addition to the instance mentioned, they described a parasite in two other cases of malaria, which they claimed was identical with the so-called Plasmodium tenue.

Craig,[3] in a criticism of Stephens' paper, stated that he had encountered several times exactly similar parasites,

Fig. I.

Fig. I.—Continued

Fig. 1.—Continued

Fig. II.

Fig. III.

in undoubted infections with Plasmodium vivax, and for this reason believed that Stephens' plasmodium was an atypical form of Plasmodium vivax, in the unpigmented stage of development. But whether or not the parasite is identical with Plasmodium vivax, it cannot be accepted as a new species until more is known of the life cycle in the human host.

Stephens,[4] in 1915, reported another peculiar malarial parasite; and in this paper acknowledged that he had overlooked the existence of Balfour's plate published in 1908 and reproduced in the paper cited above.[2] To Balfour, therefore, belongs the credit of the first description of markedly amoeboid forms of malarial parasites (other than the simple tertian). But Stephens feels that this parasite described by Balfour does not closely resemble Plasmodium tenue. However, he states, "It is of interest to record the result of an examination of further films from my case of Plasmodium tenue. Two films (March 1914) showed quartan, and in one of the films, a single pigmented (presumably single tertian) parasite was found with the pigment in the form of rods, in an enlarged cell showing Schüffner's dots. Three films (June) showed *quartan*, and one (July) *quartan*. Young ring forms were not found in any of the films."

Sinton,[5] in 1922, described a case of malaria which he thought was due to Plasmodium tenue. He stated that he had seen five such cases, and promised in a future paper to give a more detailed description of the clinical symptoms and the morphological and cultural characters of the parasites found in all of these cases.

After a review of the literature one finds that all of the patients harboring the so-called Plasmodium tenue have come from India or Africa, and that no one has as yet described a parasite resembling Plasmodium tenue in the blood of a native of this country. The object of this paper is to describe a malarial parasite resembling morphologically Plasmodium tenue, which was found in the blood of a patient who had never been out of Maryland.

CASE REPORT

B. B., negro, male, aged 35. Married, four children.

Admitted to the hospital, January 26, 1923, complaining of chills.

Family history irrelevant.

Personal History.—Has had whooping cough, mumps, and measles. In his nineteenth year he had pneumonia, malaria and a sore on the penis. He was married five years ago; there are four children living and well, and his wife has had no miscarriages. The patient has been treated in the Syphilis Clinic of this hospital since October 17, 1922, having received 0.4 gram of arsphenamin, intravenously, once a week for six weeks. The Wassermann reaction remained positive during this time.

Present Illness.—Onset two hours after the last injection of arsphenamin on December 15, 1922. The patient had a severe shaking chill lasting several hours. Since this time he had had severe chills, every second and third day, and these were followed by drenching sweats. He had noticed that exposure to cold would bring on a chill. Between chills there was no discomfort and the patient felt quite well. He had been constipated. While waiting for treatment in the Dispensary at 1.30 P. M., January 26, 1923, he was seized with a shaking chill, the temperature rose to 100.3° F., the pulse to 130, and he was admitted immediately to the hospital.

Physical Examination.—Temperature 103° F. Pulse 124, respirations 24, blood pressure 130/90.

The patient is a well nourished and well developed colored man. Height 6 feet. Weight 162 pounds. Marked sweating. Pupillary reactions normal. Perforated nasal septum. Tongue clear and of good color. General glandular enlargement. Lungs clear. Heart normal in size and position; soft blowing systolic murmur at apex but is not transmitted to the axilla. The pulse is regular, rapid and definitely dicrotic; radials palpable. Edge of spleen palpable under left costal margin on deep inspiration; otherwise abdomen normal. Scar on dorsum of penis behind sulcus. Reflexes hyperactive and equal.

Laboratory Examinations.—Urine, specific gravity 1.022, acid, albumin one plus, a few hyaline and granular casts. Stool examinations were negative for ova or parasites. The Wassermann reaction was positive; the blood examination showed a moderate grade of secondary anemia (Chart I). At 4 P. M. on January 26, 1923, two hours after the chill, the blood smear showed a moderate infection with malarial parasites which were thought to be young forms of Plasmodium falciparum. Most of the forms were "ring," and multiple infections were not uncommon. (Fig. I, line 21-22, a, b, c, d, and e). There were also a few "tenuated" forms and an occasional crescent. The ring forms were small, about one third the diameter of a red cell. At 4 P. M. the next day, January 27, 1923, the character of the parasites had changed, in that the majority of the forms found were "tenuated," and only a few of the "ring" forms could be found (Fig. I, line 2, 3, 15 and 16, a, b, c, d, and e). An occasional crescent could be found, and multiple infection was rather rare. The "rings" were larger, about two fifths the diameter of a red cell, and the protoplasm was more abundant than the chromatin (Fig. I, line 11, c; line 12, a and e; line 14, a; line 18, c). The "tenuated" parasites were thought to correspond with Stephens' Plasmodium tenue. On the third day of his stay in the hospital, January 28, 1923, very few parasites could be found and those seen were mostly "rings" although a few "tenuated" forms were present. On January 29, 1923, the fourth day after admission, the parasites had disappeared.

Course in Hospital.—The patient was put to bed on admission and kept there until the parasites had disappeared from the circulating blood, on January 29, 1923. He was then allowed to get up. Exposure to cold did not bring about a relapse. After the initial attack, the patient seemed to have no inconveniences from the infection. The temperature dropped (see Chart), although there was a slight rise each day, which did not exceed 100.2° F., and, finally, it fell gradually to normal in ten days. The patient had no more chills or chilly sensations. Quinine was not begun until January 31, 1923, the sixth day after admission.

DESCRIPTION OF THE PARASITES FOUND IN THE PERIPHERAL BLOOD

The asexual forms found may be placed in the following groups: (1) Small ring forms. (2) Large ring and oval tailed forms. (3) Band forms. (4) Tenue forms.

(1) Small ring forms were most prominent two hours after the chill and resembled the small ring forms seen in

infections with P. falciparum. They occupied about one quarter to one third the diameter of the infected red cells. Accolé forms were found but were rare. The protoplasm formed a very thin ring and the vacuole was well marked. The chromatin was not large in amount and took the form of a dot, being situated at the periphery of the parasite and bulging slightly centrally. Occasionally the chromatin was broken into parts. It was a question whether the red blood cells containing the parasites were larger or smaller than normal; but it was definitely found that where there was a double infection of the cell, the cell was always smaller. One infected cell was found which was filled with basophilic granules (Fig. III.). There was no crenation of the infected cells. Double infection of the cells was not uncommon and once four parasites were found enclosed in one cell (Fig I, line 1, b; line 9, c and e; line 11, b; line 14, e; line 21, a, b, c, d, and e; line 22, a, b, c, d, and e).

(2) Large rings and "oval tailed" forms were seen in large numbers on smears taken twenty hours after the chill. The parasites were larger and took up about two-fifths to one-half of the red blood cells. Some were large ring forms while others had tails, as seen in Fig. I, line 7, d; line 12, d. The tailed forms were the commonest, while the perfect oval forms were fewer in number. The protoplasm was larger in amount than in the small ring forms. The chromatin took on all shapes; resembling rods, bars, bands and fragmented bits. The vacuole was very distinct (Fig. I, line 2, e; line 7, d; line 10, a; line 11, a, e; line 12, a, d, e; line 13, a, b, c, d, e; line 14, a, d, e; line 16, b, d; line 17, d; line 18, c, d; line 19, d).

(3) Banded forms were seen in all the smears examined but occurred in greatest numbers in those taken from twenty to thirty hours after the chill. They resembled those seen in infection with P. malariae except that the protoplasm was much less narrow and was small in amount. The chromatin in these forms was in the shape of rods, dots, and bands, and in some was broken into pieces, while in others it seemed to have no connection with the protoplasm and was lying quite free from it (Fig. I, line 2, c; line 3, c; line 5, b; line 6, d, e; line 8, e; line 9, a; line 10, d).

(4) Tenue forms occurred in large numbers in the smears taken from twenty to thirty hours after the chill, although they were seen in all the smears from this patient,—occurring in such varied and bizarre shapes that it is difficult to describe the different forms. The protoplasm was small in amount, that is, the individual processes were thin. The chromatin occurred in various forms—rods, curves, dots, bands—and at times was broken into bits. It was abundant but very irregular in shape and form, and was scattered about irregularly (Fig. I, line 1, a, c, d, e; line 2, a; line 3, a, b, d, e; line 5, a, e; line 6, c, e; line 7, b, c, e; line 8, b; line 11, e; line 12, c; line 15, a, c, d, e; line 16, a, c, d; line 17, a, b, c, e; line 18, a, b, e; line 19, a, c, e; line 20, e).

No segmenting forms were found in any of the smears.

The sexual forms were rare but could be found in all smears. They occurred in the form of crescents, much like those seen in infections with P. falciparum. Some appeared as though breaking down, or shrinking (Fig. II).

The cycle of development could not be followed accurately, owing to the fact that the parasites disappeared from the blood so rapidly after the subsidence of symptoms. The small ring forms were the most numerous immediately after the chill. These seemed to develop into larger ring forms and tenue forms within twenty hours. In from forty-two to forty-eight hours after the chill, all the forms (rings, oval, band, and tenue) were present in about equal numbers.

BLOOD CHART I.

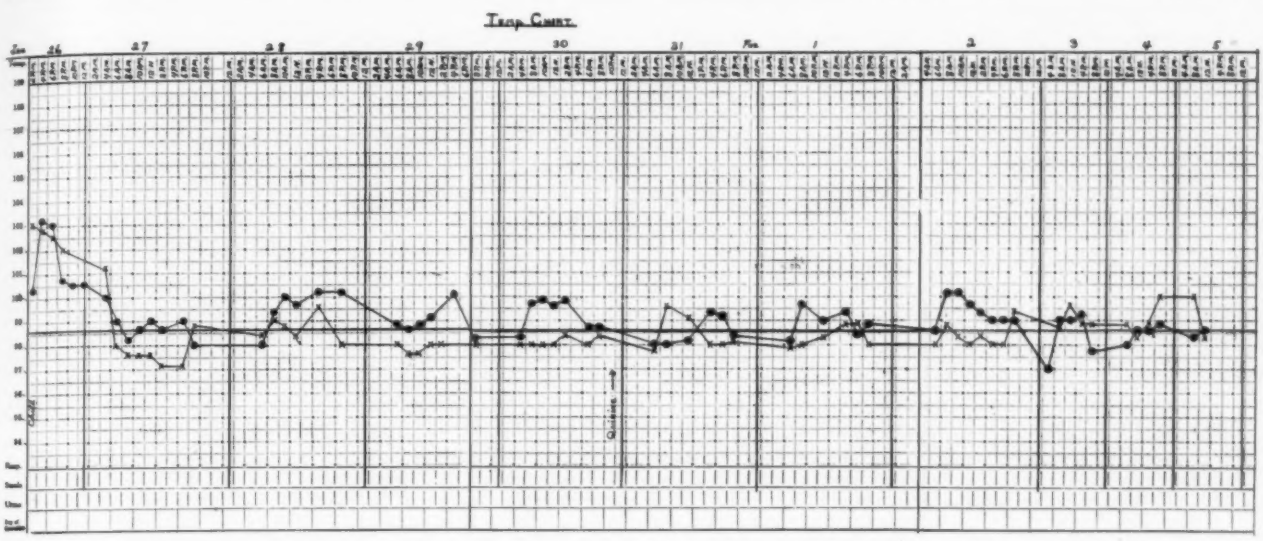
Date	1/26/23	1/31/23	2/4/23
Hb.	55%	50%	60%
R. B. C.	2,512,000	2,804,000	3,372,000
C. I.	1 plus	1 plus	1 plus
Anisocytosis	Slight	Slight	Slight
Poikilocytosis	Slight	None	None
Basophilia	Slight diffuse	Slight diffuse	None seen
Nucleated R. B. C.	None seen	None seen	None seen
W. B. C.	8,280	8,000	7,760
P. M. N.	65%	64%	69%
P. M. E.	0	1%	.2%
P. M. B.	0	0	0
L. lym.	0	0	0
S. lym.	22%	20%	26%
L. Mono.	6%	7%	0
Trans.	7%	8%	3%
Myeloblasts	None seen	None seen	None seen
Myelocytes N.	None seen	None seen	None seen
Myelocytes E.	None seen	None seen	None seen
Myelocytes B.	None seen	None seen	None seen
Path. lym.	None seen	None seen	None seen
Platelets	Normal	Normal	Increased

The blood examined forty-eight hours after the chill showed very few parasites, while in sixty hours it was free of parasites. None were seen after this time, although smears were examined daily.

The temperature chart gives no insight into the character of the infection, inasmuch as, after the initial rise, the temperature rose only slightly each day and after the ninth day remained normal.

Discussion

A parasite resembling that described by Stephens as P. tenue has been found in a colored man, aged 35. This man had never been out of the state of Maryland and the only history of a malarial infection relates to an attack



which was probably this infection sixteen years ago. He had syphilis and while being treated with arsphenamin suffered mildly with chills and fevers at irregular intervals, especially following exposure to cold. The question arises as to whether this parasite found in his blood is identical with that described by Stephens or is merely one form of the already well known types of malarial parasites. One of the infected red cells seen had basophilic stippling and there was evidence of the marked activity of the parasite. Both of these observations suggested that the parasite in question was P. vivax, although the other features characteristic of this parasite were lacking. The banded forms were suggestive of P. malariae, but the bands were not so broad as those usually seen in the latter. On the other hand, the presence of crescents in the absence of other sexual forms and the finding of red cells infected with from one to four parasites are points in favor of P. falciparum. The size of the infected red cells cannot be regarded as a deciding factor, on account of the marked variations in both directions. However, cells with multiple infections were all smaller than normal. There is the possibility, moreover, of a mixed infection with P. vivax and P. falciparum, but we are inclined to believe that this is not the explanation. P. malariae seems an improbable diagnosis, as none of these infections have been reported from this State, except in patients who had been infected in tropical countries.

Craig [3] states that "the extreme delicacy of the cytoplasmic pseudopodia seen in the so-called P. tenue is frequently observed in tertian infections in which quinine has been administered in dosage insufficient to kill the plasmodia, but enough to produce a stimulation of amoeboid activity and consequently a greater number of cytoplasmic processes. The reasons underlying the production of morphologically atypical generations of malaria plasmodia are obscure, but we are convinced that insufficient dosage with quinine, and perhaps with other drugs, often leads to the production of such plasmodia and that these plasmodia may retain for several generations morphological abnormalities produced by adverse agencies, either physical or chemical.

"In proof of this assertion we may state that we have followed infections in which the plasmodia were very atypical, being modified morphologically by the exhibition of small doses of quinine, and have seen a return to normal morphology when the drug was discontinued. One of the most common effects of quinine is a great stimulation of the amœboid activity of the malaria plasmodia by the presence of delicate pseudopodia containing an abnormal amount of chromatin during the early stages of development of the plasmodia."

Rosenburg [6] states that arsphenamin preparations frequently arouse a latent malaria, so that at the present time the typical malaria seems to be endemic in Berlin.

Fully thirty-seven per cent of all the malarial patients in the last few years, and fifty per cent with tropical malaria, had never left Germany.

The evidence indicates that the case reported in this paper may be one of chronic plasmodium falciparum infection relapsing after the administration of arsenic which stimulated the parasite to greater amœboid activity.

CONCLUSIONS

1. A malarial parasite resembling P. tenue was found in the peripheral circulation of a negro who had never been outside of the state of Maryland.

2. Should this parasite prove to be P. tenue, it is the first noted in the temperate zone.

3. From the present study it is impossible to state whether P. tenue is a new parasite or merely a form of one of the well known classified parasites of malaria.

4. The case reported may be due to (a) P. falciparum, which had been stimulated to great activity by arsphenamin, (b) may represent mixed infection of P. falciparum plus P. malariae, or (c) even a stage in the life cycle of P. vivax.

The writers wish to express their indebtedness to Drs. S. T. Darling and R. W. Hegner for examining their slides and giving them the advantages of their counsel.

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FURTHER STUDIES ON BLOOD GROUPING

IV. THE DEMONSTRATION OF TWO ADDITIONAL ISOAGGLUTININS (*D* AND *Q*) IN HUMAN BLOOD

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In earlier reports [1,2] it was announced that we had found it possible to divide twenty "Group IV" bloods into three types, distinguished by the following characteristics: *Type one* (C. E. W.), whose serum agglutinated the red cells of the second type (P. O. C.); *Type two* (P. O. C.), whose red cells were agglutinated by the serum of the first type (C. E. W.); *Type three* consisting of the eighteen others, whose sera did not agglutinate the cells of the second type (P. O. C.) and whose cells were not agglutinated by the serum of the first type (C. E. W.). The unusual agglutinin present in the cells of the first type (C. E. W.) did not agglutinate the cells of the second type (P. O. C.) at 37° C., but was active at lower temperatures; at 15° C. it caused definite clumping when diluted 1 to 16. In tests at somewhat higher temperatures the titre became less and less until at 32° C. the serum caused no agglutination of cells of the second type (P. O. C.). It was shown that a similar difference in titre at 15° and 37° C. could be demonstrated in the agglutinins present in another "Group IV" blood for two types of "Group II" cells, containing respectively agglutinogens *b* and *bc*. It was evident, therefore, that the unusual agglutinin, present in the serum of C. E. W. and capable of agglutinating the cells of P. O. C., was in relatively low concentration. As to the identity of this agglutinin we had no definite information but surmised that it was probably agglutinin *C*, whose existence we had previously discovered.[3] To determine this point we undertook rather extensive absorption experiments, using seven different human bloods.

These seven bloods were selected from a large list which had been tested against the cells and serum of one member of "Groups II" (C. G.; formula *A—bc*). By this means each had been assigned to some one of the four accepted groups, according to the possibilities outlined in Table I.[4,5]

TABLE I.

Method used for preliminary classification of bloods.

Serum	Red cells	Result			
Group II	*x*	+	0	+	0
x	Group II	0	0	+	+
Assigns *x* to		Gr. I	Gr. II	Gr. III	Gr. IV

The seven bloods used in our absorption experiments were classified by the method mentioned above, as follows: "Group I," one; "Group II," two; "Group III," one; "Group IV," three. The agglutination reactions of cells and serum from each blood were then tried with tested representatives of the four accepted groups, and, as each of the seven behaved in the traditional manner, the original classification was confirmed. When the seven bloods were cross-agglutinated by the hanging drop method and the preparations placed in the icebox at 15° C. for one and for two hours, the results were those shown in Table II.

From Table II it is seen that, with one exception, the reactions observed were according to the accepted rule; the serum of "Group IV" (C. E. W.) agglutinated the cells of "Group IV" (P. O. C.).

At this time we were already possessed of a certain amount of information concerning the agglutinin-agglutinogen content of these bloods. Inasmuch as the preliminary classification was effected by means of the reac-

TABLE II.

Results of cross-agglutination tests.

Serum	Red cells						
	Group I (M.P.)	Group II (C.G.)	Group II (J.P.)	Group III (H.A.)	Group IV (P.O.C.)	Group IV (P.J.)	Group IV (C.E.W.)
Group I (M. P.)	0	0	0	0	0	0	0
Group II (C. G.)	+	0	0	+	0	0	0
Group II (J. P.)	+	0	0	+	0	0	0
Group III (H. A.)	+	+	+	0	0	0	0
Group IV (P. O. C.)	+	+	+	+	0	0	0
Group IV (P. J.)	+	+	+	+	0	0	0
Group IV (C. E. W.)	+	+	+	+	+	0	0

tions of serum and cells of each blood with the cells and serum of a "Group II" blood the formula of which was known from previous studies (C. G.; formula *A*—*bc*), we were assured that the red cells of "Group I" (M. P.) and those of "Group III" (H. A.) contained agglutinogen *a*, and that the red cells of "Group II" (C. G.), of "Group II" (J. P.) and of the three members of "Group IV" did not contain agglutinogen *a*. In like manner we were assured that the serum of "Group III" (H. A.) and of the three members of "Group IV" contained either agglutinin *B* or agglutinin *C*, or both together, but that the serum of "Group I" (M. P.) and of the two members of "Group II" contained neither of these agglutinins. We also had some information gained from earlier studies on three of these bloods. It was known, for instance, that the two "Group II" bloods were of different types; the red cells of one of them (C. G.) contained two agglutinogens, *b* and *c*; those of the other (J. P.) contained agglutinogen *b* but not *c*; the serum of each contained agglutinin *A*. It had likewise been shown by experiment that the serum of "Group IV" (P. J.) contained agglutinins *A*, *B* and *C*. We felt also that it might be assumed as a practical certainty for each of these bloods that no agglutinogen was present in the cells which corresponded to any agglutinin present in the serum and *vice versa*. Thus, although the information which we possessed concerning these bloods prior to the absorption experiments was considerable, it was incomplete; its extent is shown in Table III.

In the absorption experiments we used each serum which, in the cross-agglutination tests, had shown the ability to clump the red cells of any member of the series; the serum was absorbed with the cells it was capable of agglutinating and the absorbed serum then tested against a fresh suspension of red cells from each of the seven bloods. The actual details consisted in the addition of 9 volumes of packed, thrice-washed cells to a tube containing 20 volumes of serum, mixing thoroughly and allowing the absorption to continue for 2 hours in the icebox at 15° C. At the end of this time each tube was centrifugated rapidly, the clear, supernatant serum was pipetted

TABLE III.

Information regarding blood formulae obtained prior to absorption experiments.

Blood	Serum agglutinins		R. B. C. agglutinogens	
	Present	Absent	Present	Absent
Group I (M. P.)		*A B C*	*a*; *b* or *c*; or *bc*	
Group II (C. G.)	*A*	*B C*	*bc*	*a*
Group II (J. P.)	*A*	*B C*	*b*	*a c*
Group III (H. A.)	*B* or *C*; or *B C*	*A*	*a*	*b* or *c*; or *b c*
Group IV (P. O. C.)	*B* or *C*; or *B C*			*b* or *c*; or *b c*
Group IV (P. J.)	*A B C*			*a b c*
Group IV (C. E. W.)	*B* or *C*; or *B C*			*b* or *c*; or *b c*

off and tested against a fresh suspension of cells from each blood in the series. Hanging-drop preparations on hollow-ground slides were set up in duplicate, two drops of serum being used to one drop of cell suspension for each test. The preparations were placed in the icebox at 15° C. and readings made after one and two hours at this temperature. The results were clear-cut and definite; they are shown in Table IV.

It may be profitable to analyze the results summarized in Table IV.

a. No suggestion of autoagglutination occurred in any of the experiments.

b. It is apparent that red cells of the type used for the preliminary absorption were not agglutinated in the subsequent tests; in other words, the absorption was complete in each instance.

c. In no instance was a serum, after absorption, capable of agglutinating cells which it was unable to agglutinate before.

d. "Group II" (C. G.) serum, when absorbed with the cells of "Group I" (M. P.) or "Group III" (H. A.), lost all power to agglutinate any cells in the series.

e. "Group II" (J. P.) serum, when absorbed with the cells of "Group III" (H. A.), behaved just as did the serum of "Group II" (C. G.); after absorption with the cells of "Group I" (M. P.), however, this serum was still capable of causing pronounced agglutination of the cells of "Group III" (H. A.).

f. "Group III" (H. A.) serum, when absorbed with the cells of either "Group I" (M. P.) or "Group II" (C. G.) lost all power to agglutinate any cells in the series; after absorption with the cells of "Group II" (J. P.), however, this serum was capable of causing pronounced agglutination of the cells of "Group I" (M. P.) and those of "Group II" (C. G.).

TABLE IV.

Results of absorption tests.

Exp. No.	Serum	Absorbed with R. B. C.	Subsequent agglutination of red cells						
			Group I (M. P.)	Group II (C. G.)	Group II (J. P.)	Group III (H. A.)	Group IV (P. O. C.)	Group IV (P. J.)	Group IV (C. E. W.)
1	Group II (C. G.)	Group I (M. P.)	0	0	0	0	0	0	0
2	Group II (C. G.)	Group III (H. A.)	0	0	0	0	0	0	0
3	Group II (J. P.)	Group I (M. P.)	0	0	0	+	0	0	0
4	Group II (J. P.)	Group III (H. A.)	0	0	0	0	0	0	0
5	Group III (H. A.)	Group I (M. P.)	0	0	0	0	0	0	0
6	Group III (H. A.)	Group II (C. G.)	0	0	0	0	0	0	0
7	Group III (H. A.)	Group II (J. P.)	+	+	0	0	0	0	0
8	Group IV (P. O. C.)	Group I (M. P.)	0	0	0	0	0	0	0
9	Group IV (P. O. C.)	Group II (C. G.)	+	0	0	+	0	0	0
10	Group IV (P. O. C.)	Group II (J. P.)	+	+	0	+	0	0	0
11	Group IV (P. O. C.)	Group III (H. A.)	+	+	+	0	0	0	0
12	Group IV (P. J.)	Group I (M. P.)	0	0	0	0	0	0	0
13	Group IV (P. J.)	Group II (C. G.)	+	0	0	+	0	0	0
14	Group IV (P. J.)	Group II (J. P.)	+	+	0	+	0	0	0
15	Group IV (P. J.)	Group III (H. A.)	+	+	+	0	0	0	0
16	Group IV (C. E. W.)	Group I (M. P.)	0	0	0	0	+	0	0
17	Group IV (C. E. W.)	Group II (C. G.)	+	0	0	+	+	0	0
18	Group IV (C. E. W.)	Group II (J. P.)	+	+	0	+	+	0	0
19	Group IV (C. E. W.)	Group III (H. A.)	+	+	+	0	+	0	0
20	Group IV (C. E. W.)	Group IV (P. O. C.)	+	+	+	+	0	0	0
21	Group IV (C. E. W.)	Group IV (P. J.)	+	+	+	+	+	0	0

g. The sera of "Group IV" (P. O. C.) and of "Group IV" (P. J.) behaved alike throughout. Absorption with the cells of "Group I" (M. P.) caused a loss of all agglutinative power for any cells in the series; absorption with the cells of "Group II" (C. G.) caused a loss of agglutinative power for these cells and for those of "Group II" (J. P.), but not for those of "Group I" (M. P.) or "Group III" (H. A.); absorption with the cells of "Group II" (J. P.) caused a loss of agglutinative power for these cells but not for those of "Group I" (M. P.), "Group II" (C. G.) or "Group III" (H. A.); absorption with the cells of "Group III" (H. A.) did not affect the agglutinative power for the cells of "Group I" (M. P.), "Group II" (C. G.) or "Group II" (J. P.).

h. With one exception, the serum of "Group IV" (C. E. W.) behaved as did the sera of "Group IV" (P. O. C.) and "Group IV" (P. J.). The cells of "Group IV" (P. O. C.) were the only ones in the series capable of removing from the serum of "Group IV" (C. E. W.) the agglutinin for the cells of "Group IV" (P. O. C.); the only agglutinin removed by absorption of the serum of "Group IV" (C. E. W.) with the cells of "Group IV" (P. O. C.) was that for the cells of "Group IV" (P. O. C.); absorption with the cells of "Group IV" (P. J.) removed no agglutinin whatever.

It is thus seen that the cells of the two representatives of "Group II" did not behave alike. When used to absorb the serum of "Group III" (H. A.), "Group IV" (P. O. C.), "Group IV" (P. J.) and "Group IV" (C. E. W.), the cells of "Group II" (C. G.) removed the agglutinin for those cells and that for the cells of "Group II" (J. P.) as well (Experiments 6, 9, 13 and 17); when the cells of "Group II" (J. P.) were used to absorb the same sera, the agglutinin for those cells was removed but not that for the cells of "Group II" (C. G.) (Experiments 7, 10, 14 and 18). This can be explained by assuming that there was one agglutinogen (*b*) possessed by each of these types of "Group II" cells and an additional agglutinogen (*c*) possessed by the cells of "Group II" (C. G.) but not by those of "Group II" (J. P.). This interpretation would also indicate the presence of agglutinins corresponding to both of these agglutinogens in the serum of "Group III" (H. A.) and the three representatives of "Group IV."

These observations were confirmed by the results of agglutination tests upon "Group I" (M. P.) cells by the serum of "Group III" (H. A.). After absorption with the cells of "Group II" (J. P.), agglutination of the cells of "Group I" (M. P.) was present; after absorption with the cells of "Group II" (C. G.) it was absent (Experi-

ments 6 and 7). This was taken to indicate the presence of both agglutinogens *b* and *c* in the cells of "Group I" (M. P.) and of the corresponding agglutinins in the serum of "Group III" (H. A.).

After absorption of any of the three "Group IV" sera with either type of "Group II" cells, agglutinin was still present for the cells of "Group I" (M. P.) (Experiments 9, 10, 13, 14, 17 and 18). This showed that there was present in the cells of "Group I" (M. P.) an agglutinogen (*a*) other than *b* or *c* and that the corresponding agglutinin was present in the sera of all three representatives of "Group IV," an agglutinin not present in the serum of "Group III" (H. A.).

The three representatives of "Group IV" behaved alike in their reactions to the cells of "Group III" (H. A.); when absorbed with these cells, agglutinin for them was lost but that for the cells of "Group I" (M. P.) and both types of "Group II" cells was retained (Experiments 11, 15 and 19); when absorbed with the cells of either type of "Group II" cells, the agglutinin for the cells of "Group III" was retained (Experiments 9, 10, 13, 14, 17 and 18); when absorbed with the cells of "Group I" (M. P.), the agglutinin for the cells of "Group III" (H. A.) was lost (Experiments 8, 12 and 16). These reactions showed (1) that there was present in the cells of "Group III" (H. A.) an agglutinogen (*a*) other than *b* or *c*; (2) that this agglutinogen (*a*) was likewise present in the cells of "Group I" (M. P.); (3) that it was absent from the cells of either "Group II" blood, and (4) that the corresponding agglutinin was present in the serum of each of the three representatives of "Group IV."

Absorption of any of the three "Group IV" sera with the cells of "Group I" (M. P.) removed all of the agglutinin for these cells and also for the cells of "Group III" (H. A.) and both types of "Group II" (Experiments 8, 12 and 16). This was taken to indicate the presence in the cells of "Group I" (M. P.) of agglutinogens *a*, *b* and c and in the "Group IV" sera of the corresponding agglutinins (*A*, *B* and *C*).

Since absorption of the serum of "Group III" (H. A.) by the cells of "Group I" (M. P.) left no agglutinin for any cells in the series (Experiment 5) it was considered as confirmed that there were present in the cells of "Group I" (M. P.) the agglutinogens (*bc*) corresponding to the agglutinins (*B C*) present in the serum of "Group III" (H. A.).

In cross-agglutination tests the sera of the two members of "Group II" gave just the same reactions; each agglutinated the cells of "Group I" (M. P.) and of "Group III" (H. A.) but of no other members of the series. After absorption with the cells of "Group III" (H. A.) no blood was agglutinated by either serum (Experiments 2 and 4). After absorption with the cells of "Group I" (M. P.), however, they no longer behaved alike; the serum of "Group II" (C. G.) had lost all agglutinin for any blood in the series, but the serum of "Group II" (J. P.) strongly agglutinated the cells of "Group III" (H. A.) (Experiments 1 and 3). This showed plainly (1) that there was at least one agglutinin (*A*) for the cells of "Group III" (H. A.) possessed by both types of "Group II" serum; (2) that the serum of "Group II" (J. P.) contained another agglutinin (*D*) for the cells of "Group III" (H. A.); (3) that this agglutinin (*D*) was not present in the serum of "Group II" (C. G.); (4) that the agglutinogen (*d*) corresponding to this unusual agglutinin was not present in the cells of "Group I" (M. P.) and so was an agglutinogen other than *a*, *b* or *c*; (5) that the cells of "Group III" (H. A.) contained agglutinogen (*a*) corresponding to the agglutinin (*A*) in the serum of both representatives of "Group II" and also the other agglutinogen (*d*) corresponding to the extra agglutinin (*D*) in the serum of "Group II" (J. P.). It was also clear that this new agglutinin (*D*) was present in only one of the seven sera and the corresponding agglutinogen (*d*) in only one of the seven sets of cells in the series.

As the bloods of the two representatives of "Group II" did not agglutinate each other, we were now assured that the serum of "Group II" (J. P.) did not contain agglutinins *B* or *C*. Since the cells of none of the three representatives of "Group IV" were agglutinated by the serum of "Group III" (H. A.) or that of either type of "Group II," these cells evidently did not contain agglutinogens *a*, *b*, *c* or *d*.

In the cross-agglutination tests, it was seen that the cells of "Group IV" (P. O. C.) were agglutinated by the serum of "Group IV" (C. E. W.) and in the absorption tests this observation was confirmed. Absorption of this serum by those cells removed this agglutinin but no agglutinin for any other cells in the series (Experiment 20). By absorption with the cells of "Group I" (M. P.), "Group III" (H. A.) and both types of "Group II," the same reactions were obtained as when the other "Group IV" bloods were absorbed with these same cells but in no instance did this affect the agglutinin for the cells of "Group IV" (P. O. C.) (Experiments 16, 17, 18 and 19). Absorption of the serum of "Group IV" (C. E. W.) with the cells of "Group IV" (P. J.) did not affect the agglutinin content (Experiment 21). From these results it was learned that the agglutination of the cells of "Group IV" (P. O. C.) by the serum of "Group IV" (C. E. W.) was dependent upon the reaction of an agglutinin other than *A*, *B*, *C*, or *D* with an agglutinogen other than *a*, *b*, *c* or *d*. Inasmuch as this agglutinin (1) was present in much lower concentration than the isoagglutinins previously recognized, so low, in fact, as to necessitate a temperature below that of the body for its demonstration, and (2) was active against the cells of another member of the same accepted group, we would suggest for its designation the letter *Q*, as being less apt to cause confusion concerning its actual nature when this is finally

determined than might arise from the use of number, a Greek letter or the letter next in alphabetical sequence after those used to designate the other isoagglutinins of a possibly different nature; the corresponding isoagglutinogen we would label *q*. This agglutinin was found in the serum of only one of the seven sera and the corresponding agglutinogen in only one of the seven sets of cells in the series.

As was stated above in speaking of the information available prior to the performance of these absorption experiments, we consider that one is justified in assuming that an agglutinin and the agglutinogen which corresponds to it are not present in the same blood, and may draw the natural conclusions in arriving at the agglutinin-agglutinogen content of these seven bloods. The information concerning the formulae of these bloods which was collected from the results of the absorption experiments is shown in Table V.

TABLE V.

Information regarding blood formulae obtained from absorption experiments.

Blood	Serum agglutinins		R. B. C. agglutinogens	
	Present	Absent	Present	Absent
Group I (M. P.)		*ABCDQ*	*abc*	*dq*
Group II (C. G.)	*A*	*BCDQ*	*bc*	*adq*
Group II (J. P.)	*A D*	*B C Q*	*b*	*acdq*
Group III (H. A.)	*B C*	*A D Q*	*ad*	*bcq*
Group IV (P. O. C.)	*A B C*	*D Q*	*q*	*abcd*
Group IV (P. J.)	*A B C*	*D Q*		*abcdq*
Group IV (C. E. W.)	*ABCQ*	*D*		*abcdq*

Comment

A comparison of Table III with Table V shows that the knowledge concerning these bloods obtained from the absorption experiments has confirmed in detail the information derived by other means before the absorption tests were made but has also added certain facts of importance.

The absorption tests were repeated throughout—some of the crucial ones several times—without changing the results. It had been planned to include in our series the blood of "Group II" (H.) and of "Group II" (D. J.) on which had been made rather thorough studies mentioned in earlier reports.[3,6] These bloods were secured from Baltimore but did not reach us in time to be included in the series; when they did arrive, two of the most important members of our series—"Group I" (M. P.) and "Group III" (H. A.)—were no longer available, having left for their summer vacation; and the cells originally obtained from the seven persons in our series were so old as to be utterly unfit for use. The absorbed sera, however, were still on hand and with these it was possible to test the cells of the two additional bloods. At this time, consequently, we cannot report further on the behavior of the sera of these two bloods, but the cells of "Group II" (D. J.) behaved like those of "Group II" (C. G.), the cells of "Group II" (H.) like those of "Group II" (J. P.).

To these observations may be added the results of another series of absorption experiments with the sera of six bloods not previously used in our tests—one member of "Group III" and five members of "Group IV." Absorption of any of these sera with the cells of either "Group II" (C. G.) or "Group II" (D. J.) removed all of the agglutinin for these two bloods and also for the other pair. Absorption with the cells of either "Group II" (J. P.) or "Group II" (H.), however, completely removed the agglutinin for both of these bloods but left powerful agglutinin for the cells of the first pair. Such results merely served to confirm our previous impression that these four "Group II" bloods were of two distinct types, the red cells of one type (C. G. and D. J.) containing two agglutinogens (*bc*), the red cells of the other type (H. and J. P.), only one (*b*).

It has been noted above that the bloods of "Group II" (C. G.) and "Group II" (J. P.) differed not only in their agglutinogen, but also in their agglutinin content. Whether the presence of the extra agglutinin is a distinguishing characteristic of all "Group II" bloods which lack the extra agglutinogen, and whether the extra agglutinogen always occurs in the members of this group which lack the extra agglutinin, we are unable to say at present; we plan to investigate these points in the near future.

In an earlier communication,[3] it was stated that agglutinogen *c* was found in the erythrocytes of neither of the two representatives of "Group I" studied at that time; the formula assigned to each of these bloods was *O—ab*. In the experiments reported in the present paper, however, it was found that the cells of "Group I" (M. P.) contained agglutinogens *a*, *b* and *c;* this makes three "Group I" bloods—one before and one since the tests with M. P.—which we have found with the formula *O—abc*. Which is the commoner of these two types cannot be determined from so small a series. It is interesting to note that agglutinogen *d* was not present in the cells of "Group I" (M. P.) nor in those of the one member of that group since tested; whether it was present in the cells of those tested earlier we do not know.

To the fact that agglutinogen *d* was absent from the cells of "Group I" (M. P.) we owe the opportunity of demonstrating its presence in those of "Group III" (H. A.); had it been present in both we should have been unable to recognize it in either. That this agglutinogen is not uncommonly associated with agglutinogen *a* in the cells of "Group III" bloods, is shown by the fact that, in some later work, we have found the two together in the erythrocytes of six members of this group.

The three "Group IV" bloods in the present series were readily separated into three types, depending on the presence or absence of agglutinin *Q* and of the corresponding agglutinogen. As was mentioned in another

place,[2] we cannot state with assurance that this agglutinin and this agglutinogen (*Q; q*) which act *within* one of the accepted groups are of exactly the same nature as the others described (*A, B, C* and *D; a, b, c* and *d*) which are operative *between* the accepted groups. The other point of difference lies in the strength of the agglutinins, agglutinin *Q* being in considerably lower concentration than the others and consequently requiring a lower temperature for its demonstration. Qualitatively, the agglutination produced was quite as definite and the action quite as specific as that effected by any of the other agglutinins mentioned.

The results obtained in these experiments were rendered possible because our series included more than one representative of some of the accepted groups. These representatives—as, for instance, the two members of "Group II"—were selected because they previously had been found to differ from each other in some respect. If one were to make the same kind of experiments with a single representative from each of the four accepted groups—let us say, with "Group I" (M. P.), "Group II" (C. G.), "Group III" (H. A.) and any one of the three members of "Group IV"—the results would apparently confirm the accepted theory of the existence of only two agglutinins and two agglutinogens (Experiments 1, 2, 5, 6, 8, 9 and 11).

Summary and Conclusions

1. Our earlier observations concerning the existence in human blood of *at least three* isoagglutinins and the corresponding isoagglutinogens have been confirmed and extended.

2. A fourth isoagglutinin (*D*) has been demonstrated in the serum of one member of "Group II" in association with isoagglutinin *A*. Isoagglutinin *D* was not found in the serum of any other blood in the series including one member of "Group I," another member of "Group II," one member of "Group III" and three members of "Group IV."

3. The corresponding isoagglutinogen (*d*) has been demonstrated in the red cells of one member of "Group III." It was not present in the six other bloods in the series but has since been found in the erythrocytes of six additional members of "Group III" in association with isoagglutinogen *a*.

4. The presence of isoagglutinogen *c* in association with isoagglutinogens *a* and *b* has been demonstrated in the red cells of three members of "Group I." In an earlier investigation two members of this group were found whose red cells contained isoagglutinogens *a* and *b* but not isoagglutinogen *c*.

5. A fifth isoagglutinin (*Q*) was found in the serum of one member of "Group IV;" it was quite distinct from agglutinins *A, B, C* and *D*. The corresponding isoagglutinogen (*q*) was present in the red cells of another member of "Group IV." Neither this agglutinin nor this agglutinogen were found in any other blood in the series. This agglutinin (*Q*) was present in lower concentration than the others and therefore required a lower temperature for its demonstration. Its action occurred *within* one of the four accepted groups rather than *between* these groups but in the quality and specificity of the agglutination produced, it was in no way inferior to the others.

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CORRECTION

In Dr. Johnstone's article in the March Bulletin, paragraph 6 on page 87, the first sentence which reads "Caudling the egg proved to be the most satisfactory way of determining the position of the embryo" should read "Candling the egg, etc."

The Johns Hopkins Hospital Bulletins are issued monthly. They are printed by MEYER & THALHEIMER, Baltimore. Subscriptions, $4.00 a year (foreign postage, 50 cents), may be addressed to the publishers, THE JOHNS HOPKINS PRESS, BALTIMORE; single copies will be sent by mail for fifty cents each.

NOTES ON NEW BOOKS

A Textbook of Anatomy and Physiology for Schools of Nursing, Normal Schools and Colleges. By JESSE FEIRING WILLIAMS, M. D. 523 pages with 369 illustrations of which 25 are in colors. $3.00. *(W. B. Saunders Co., Philadelphia & London, 1923.)*

This book is a distinct departure from the commonly used textbook of anatomy and physiology for nurses. Of the 523 pages only about a half are devoted to the text, the numerous illustrations and tables occupying the remainder of the volume. With the reading matter thus restricted, the problem of what to include and what to exclude became correspondingly difficult; yet, on the whole, the author has met this issue in a very satisfactory manner. Obviously, points of difference regarding the subject matter naturally arise, and one might ask whether students should be expected to know what percentage of dry substance is added to the embryo in the last three months of intra-uterine life, or what chalones are; it would seem better for them to know more about the physiology of the thyroid gland than that given in a brief footnote, and more important still would be the knowledge of a method for artificial respiration. However, the book avoids to a great extent the disproportionate treatment of anatomy and physiology at the expense of the latter which usually characterizes works of this nature. A notable feature is the exceptionally large amount of space devoted to embryology. Each chapter has at its beginning an outline, and at its end numerous helpful practical exercises and questions; furthermore, there is appended a good reference list for further study. However, it is the illustrations that add particular freshness and value to the book. One is glad to find, in place of hackneyed pictures handed down from textbook to textbook, numerous well-chosen new and, at times, original illustrations, which compensate to a great degree for the retrenchment in the text. The author and publisher are to be commended for having produced a book which marks a definite advance in the sphere for which this volume is intended. F. C. L.

The Heart in Modern Medicine. By WILLIAM DUNCAN REID. *(Philadelphia and London, J. B. Lippincott Co.)*

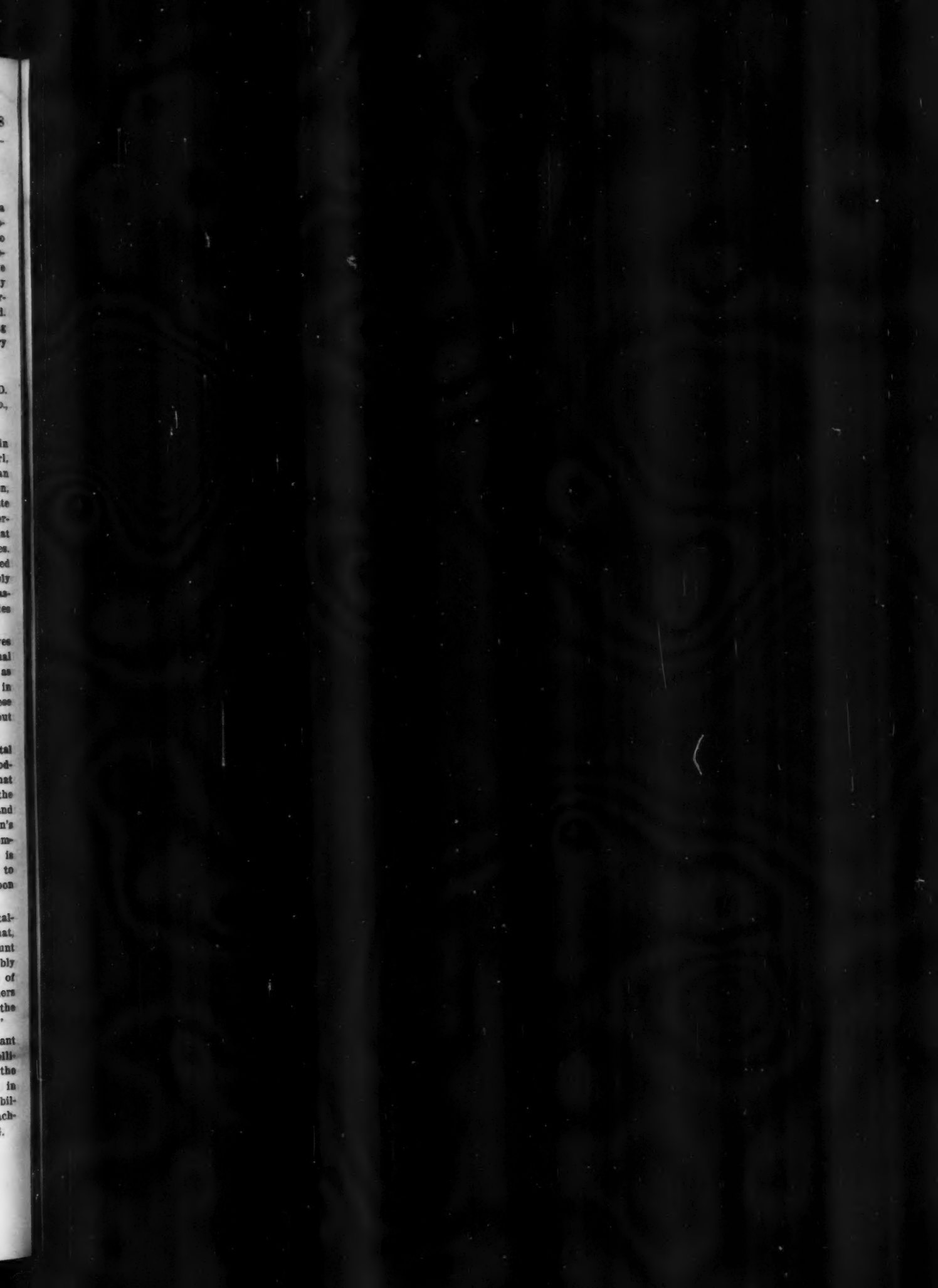
In his preface, Dr. W. D. Reid explains his purpose—to supply a book "which will incorporate the best of the new knowledge with that less recently acquired, but which may be said to have stood the test of time." He believes "such a book should preferably be brief enough to be attractive to the bulk of the profession, whose opportunity to read upon a single aspect of medicine is limited." It would seem that his book fully justifies his effort. It presents in a sane and delightful manner the present story of cardiology. There is very little new in the book, and for one who is daily following the advance in this specialty of medicine, it will serve only as a light review. By the general practitioner and by those engrossed in such work as surgery, however, it could be most advantageously read. They would gain a concise knowledge of the relationship of the heart to their specialty, and would learn to apppreciate the indications for the use of a "heart station" with its laboratory procedures. The subject is presented in a somewhat different manner from that of the ordinary book on this subject, in that the recognition of the etiological type of cardiac disease is made essential, and the greater importance of functional condition over anatomical lesion is emphasized. The chapter on "Treatment" emphasizes the need of employing graduated and controlled exercise as a therapeutic procedure in the majority of cases of cardiac disease, and shows that digitalis is only an adjunct in therapy to be employed only on the appearance of certain definite symptoms. Nearly a fifth of the book is an appendix of illustrative case reports, which are of little interest, so that there are only two hundred and seventy-five pages of large type, interspersed with a few fair photographs and tracings, to be read. The book is not at all technical, and will provide an evening or two of pleasure for those who are interested in summary presentations of specialized medicine. E. W. B.

The Action of Alcohol on Man. By ERNEST H. STARLING, M.D. $4.50. *(London and New York. Longmans, Green and Co., 1923.)*

Alcohol, "consolatio ultima corporis humani," has found, in Dr. Starling and his collaborators, Hutchison, Mott and Pearl, a group of able defenders. Its uses as a food, action on human behavior, influence on digestion, effects on blood circulation, respiration, and body temperature, are treated in separate chapters, while others devoted to the effects of its immoderate use, and its influence upon the community, conclude that portion of the book which Dr. Starling himself contributes. His opinion on the last named subject may well be illustrated by a quotation (p. 166) "Moreover, alcoholic drinks supply the most widespread and easily obtainable means of increasing the pleasures of life and diminishing the frets and worries of daily existence."

"Wine is the milk of old age," and Robert Hutchison gives a short account of other uses of alcohol from a medicinal standpoint. He discusses its functions as a food in disease, as an aid to digestion, as a tonic, sedative, and hypnotic, and in disorders of the circulation and heart. For all of these purposes he finds its employment not only legitimate but desirable.

Sir Frederick Mott, in the section on "Alcohol and Mental Disorders" states in his concluding paragraph that, "Its moderate use—may act beneficially by tending to remove that cautious self-criticism and selfishness which restrains the natural and spontaneous feelings of human sympathy and sociability which spring from the affective side of man's nature." And later (quoting Dr. Stewart Paton) "In communities when the spirit of prohibition is active, there is almost always an unusual amount of nervousness, not to mention an exceedingly defective and unhealthy outlook upon human problems."

In the last section of the book, (on "Alcohol and Mortality") Pearl concludes his contribution with the statement that, ".....the consumption of alcoholic liquors up to an amount —in common parlance called moderate—does not sensibly shorten the mean duration of life or increase the rate of mortality as compared with that enjoyed by total abstainers from alcohol." But—"excessive use of alcohol diminishes the mean duration of life and increases the rate of mortality."

It is to be doubted whether this book will have an important effect upon public opinion in the United States. The intelligentsia in general are already familiar with the facts, and the remainder of the population, great in numbers but small in understanding, have neither the desire to hear nor the ability to digest scientific information at variance with the teachings of the spellbinders who sway their emotions. C. H. G.